

6140552

sau96I
 haeIII
 asuI
 sau96I
 nlaIV
 hgiIII
 ecoII109I
 bsp1286
 banII
 asuI
 nlaIV
 mnlI
 mboII
 mnlI
 auaI
 apaI
 mnlI
 tth111I
 mnlI
 ecoII109I
 1 GGA CTT GTC TTC CTC GTC CTG CTG TTC CTC GGG GCC CTC GGA CTG
 -18 Gly Leu Val Phe Leu Val Leu Leu Phe Leu Gly Ala Leu Gly Leu
 haeIII
 eaeI
 cfrI
 hinPI
 hhaI
 46 TGT CTG GCT GGC CGT AGG AGA AGG AGT GTT CAG TGG TGC GCC GTA TCC
 -3 Cys Leu Ala Gly Arg Arg Arg Arg Ser Val Gln Trp Cys Ala Val Ser
 haeIII
 mnlI
 auaI
 haeI
 94 CAA CCC GAG GCC ACA AAA TGC TTC CAA TGG CAA AGG AAT ATG AGA AAA
 14 Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys
 mnlI
 sau96I
 haeIII
 asuI
 fnu4HI
 bbvI
 aluI
 pvuII
 pleI
 hinfI
 bsmAI
 fokI
 bsrI
 142 GTG CTG GGC CCT CCT GTC AGC TGC ATA AAG AGA GAC TCC CCC ATC CAG
 30 Val Arg Gly Pro Pro Val Ser Cys Ile Lys Arg Asp Ser Pro Ile Gln
 haeIII
 haeI
 scrFI
 ecoRII
 bstNI
 haeIII
 sau96I
 asuI
 sfaNI
 190 TGT ATC CAG GCC ATT GCG GAA AAC AGG GCC GAT GCT GTG ACC CTT GAT
 46 Cys Ile Gln Ala Ile Ala Glu Asn Arg Ala Asp Ala Val Thr Leu Asp
 sau96I
 nlaIV
 scrFI
 ecoRII
 bstNI
 haeIII
 stuI
 haeIII
 mnlI
 haeI
 asuI
 238 GGT GGT TTC ATA TAC GAG GCA GGC CTG GCC CCC TAC AAA CTG CGA CCT
 62 Gly Gly Phe Ile Tyr Glu Ala Gly Leu Ala Pro Tyr Lys Leu Arg Pro

FIG.-1A

sau96I
 auaII
 asuI
 fnu4HI accI nlaIV
 286 GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT
 78 Val Ala Ala Glu Val Tyr Gly Thr Glu Arg Gln Pro Arg Thr His Tyr

fnu4HI
 mboII bbvI aluI
 hphI fnu4HI aluI pvuII
 334 TAT GCC GTG GCT GTG GTG AAG AAG GGC GGC AGC TTT CAG CTG AAC GAA
 94 Tyr Ala Val Ala Val Val Lys Lys Gly Gly Ser Phe Gln Leu Asn Glu

haeIII sau96I
 stuI auaII
 bglI haeI asuI foki
 382 CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA
 110 Leu Gln Gly Leu Lys Ser Cys His Thr Gly Leu Arg Arg Thr Ala Gly

sau96I
 auaII
 asuI
 nlaIV
 430 TGG AAT GTC CCT ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT
 126 Trp Asn Val Pro Thr Gly Thr Leu Arg Pro Phe Leu Asn Trp Thr Gly

hgiIII aluI
 bsp1286 fnu4HI
 banII bbvI ddeI aluI
 ddeI mnlI pvuII mboII pvuII
 478 CCA CCT GAG CCC ATT GAG GCA GCT GTG CAG TTC TTC TCA GCC AGC TGT
 142 Pro Pro Glu Pro Ile Glu Ala Ala Val Gln Phe Phe Ser Ala Ser Cys

mspI
 hpaII
 scrFI
 nciI
 cauII
 526 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT
 158 Val Pro Gln Ala Asp Lys Gly Gln Phe Pro Asn Leu Cys Arg Leu Cys

nlaIV
 scrFI
 ecoRII
 mnlI bstNI rsaI
 574 GCG GCG ACA GGG GAA AAC AAA TGT GCC TTC TCC TCC CAG GAA CCG TAC
 174 Ala Gly Thr Gly Glu Asn Lys Cys Ala Phe Ser Ser Gln Glu Pro Tyr

FIG.-1B

nlaIV
 hgiCI
 aluI banI ddeI bsmal bsmal
 622 TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC
 190 Phe Ser Tyr Ser Gly Ala Phe Lys Cys Leu Arg Asp Gly Ala Gly Asp

sau96I
 avall
 asuI
 ppuMI
 ecoD109I
 hgiAI
 bsp1286 mnlI mnlI
 670 GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT GAG GAC CTG TCA GAC GAG
 206 Val Ala Phe Ile Arg Glu Ser Thr Val Phe Glu Asp Leu Ser Asp Glu

718 GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG
 222 Ala Glu Arg Asp Glu Tyr Glu Leu Leu Cys Pro Asp Asn Thr Arg Lys

scrFI
 nciI
 mspI
 hpall
 caull
 xmaI sau96I
 smaI nlaIV
 scrFI
 nciI avall
 caull
 avall asuI
 sau96I ppuMI
 haeIII nlaIV
 bsrI asuI ecoD109I nlaIII
 766 CCA GTG GAC AAG TTC AAA GAC TGC CAT CTG GCC CGG GTC CCT TCT CAT
 238 Pro Val Asp Lys Phe Lys Asp Cys His Leu Ala Arg Val Pro Ser His

bglI draIII foki mnlI hinfI mboII
 814 GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT
 254 Ala Val Val Ala Arg Ser Val Asn Gly Lys Glu Asp Ala Ile Trp Asn

scrFI
 ecoRII
 bstNI hphI
 862 CTT CTC CGC CAG GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA
 270 Leu Leu Arg Gln Ala Gln Glu Lys Phe Gly Lys Asp Lys Ser Pro Lys

FIG.-1C

sau3AI
 mboI
 dpnI
 xhoII
 bstYI
 bglII
 aluI
 bstXI
 nlaIV
 910 TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG
 286 Phe Gln Leu Phe Gly Ser Pro Ser Gly Gln Lys Asp Leu Leu Phe Lys
 nlaIV
 hgiCI
 pleI
 hinfI
 mnlI bsp1286 mnlI
 taqI banI auaI hinfI
 958 GAC TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG
 302 Asp Ser Ala Ile Gly Phe Ser Arg Val Pro Pro Arg Ile Asp Ser Gly
 mspI
 styl
 hpaII
 rsaI
 nlaIV
 fokI
 mnlI
 1006 CTG TAC CTT GGC TCC GGC TAC TTC ACT GCC ATC CAG AAC TTG AGG AAA
 318 Leu Tyr Leu Gly Ser Gly Tyr Phe Thr Ala Ile Gln Asn Leu Arg Lys
 mspI
 hpaII
 thaI
 scrFI
 fnuDII
 nciI
 bstUI
 mnlI
 fnu4HI
 hinPI
 mnlI
 bbvI cauII
 hhaI
 1054 AGT GAG GAG GAA GTG GCT GCC CGG CGT GCG CGG GTC GTG TGG TGT GCG
 344 Ser Glu Glu Glu Val Ala Ala Arg Arg Ala Arg Val Val Trp Cys Ala
 hinPI
 mstI
 fspI
 fnu4HI
 aluI hhaI
 bstXI
 bsrI
 alwNI bbvI
 1102 GTG GGC GAG CAG GAG CTG CGC AAG TGT AAC CAG TGG AGT GGC TTG AGC
 350 Val Gly Glu Gln Glu Leu Arg Lys Cys Asn Gln Trp Ser Gly Leu Ser
 fnu4HI
 mnlI
 bbvI
 bspMI
 mnlI haeIII
 mnlI
 sfaNI
 1150 GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC
 366 Glu Gly Ser Val Thr Cys Ser Ser Ala Ser Thr Thr Glu Asp Cys Ile
 scrFI
 ecoRII
 bstNI
 bstXI
 aluI sfaNI nlaIII
 fokI
 mnlI
 1198 GCC CTG GTG CTG AAA GGA GAA GCT GAT GCC ATG AGT TTG GAT GGA GGA
 382 Ala Leu Val Leu Lys Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly

FIG.-1D

nlaIII nlaIV scrFI
 sphi hgiCI ecorII
 rsal nspCIx banI bstNI
 1246 TAT GTG TAC ACT GCA TGC AAA TGT GGT TTG GTG CCT GTC CTG GCA GAG
 398 Tyr Val Tyr Thr Ala Cys Lys Cys Gly Leu Val Pro Val Leu Ala Glu

sau3AI
 mboI
 dpnI
 alwI
 1294 AAC TAC AAA TCC CAA CAA AGC AGT GAC CCT GAT CCT AAC TGT GTG GAT
 414 Asn Tyr Lys Ser Gln Gln Ser Ser Asp Pro Asp Pro Asn Cys Val Asp

sau3AI
 mboI
 dpnI
 ecorI ecorV
 1342 AGA CCT GTG GAA GGA TAT CTT GCT GTG GCG GTG GTT AGG AGA TCA GAC
 430 Arg Pro Val Glu Gly Tyr Leu Ala Val Ala Val Val Arg Arg Ser Asp

scrFI
 ecorII
 bstNI
 1390 ACT AGC CTT ACC TGG AAC TCT GTG AAA GGC AAG AAG TCC TGC CAC ACC
 446 Thr Ser Leu Thr Trp Asn Ser Val Lys Gly Lys Lys Ser Cys His Thr

haeIII
 nlaIII
 styl sau96I mboII
 ncoI asuI earI
 pstI
 1438 GCC GTG GAC AGG ACT GCA GGC TGG AAT ATC CCC ATG GGC CTG CTC TTC
 462 Ala Val Asp Arg Thr Ala Gly Trp Asn Ile Pro Met Gly Leu Leu Phe

nlaIV
 hgiII
 bsp1286
 banII sspl aluI bsp1286
 1486 AAC CAG ACG GGC TCC TGC AAA TTT GAT GAA TAT TTC AGT CAA AGC TGT
 478 Asn Gln Thr Gly Ser Cys Lys Phe Asp Glu Tyr Phe Ser Gln Ser Cys

sau3AI
 mboI
 DpnI
 scrFI xhoII
 ecorII bstYI
 bstNI auaI bglII hgiAI
 bsp1286
 1534 GCC CCT GGG TCT GAC CCG AGA TCT AAT CTC TGT GCT CTG TGT ATT GGC
 484 Ala Pro Gly Ser Asp Pro Arg Ser Asn Leu Cys Ala Leu Cys Ile Gly

FIG.-1E

hphI bsp1286
 1582 GAC GAG CAG GGT GAG AAT AAG TGC GTG CCC AAC AGC AAT GAG AGA TAC
 510 Asp Glu Gln Gly Glu Asn Lys Cys Val Pro Asn Ser Asn Glu Arg Tyr

nlaIV
 hgiCI
 banI scrFI
 mspI ecoRII
 bsrI hpaII bstNI ddeI bsmI bsmal
 1630 TAC GGC TAC ACT GGG GCT TTC CGG TGC CTG GCT GAG AAT GCT GGA GAC
 526 Tyr Gly Tyr Thr Gly Ala Phe Arg Cys Leu Ala Glu Asn Ala Gly Asp

1678 GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT
 542 Val Ala Phe Val Lys Asp Val Thr Val Leu Gln Asn Thr Asp Gly Asn

fnu4HI
 bbvI
 hinPI
 hhaI
 mnlI nlaIII ddeI aluI
 1726 AAC AAT GAG GCA TGG GCT AAG GAT TTG AAG CTG GCA GAC TTT GCG CTG
 558 Asn Asn Glu Ala Trp Ala Lys Asp Leu Lys Leu Ala Asp Phe Ala Leu

taqI
 mnlI bglI mnlI ddeI fnu4HI
 bbvI
 aluI
 1774 CTG TGC CTC GAT GGC AAA CGG AAG CCT GTG ACT GAG GCT AGA AGC TGC
 574 Leu Cys Leu Asp Gly Lys Arg Lys Pro Val Thr Glu Ala Arg Ser Cys

sau96I
 nlaIV
 nlaIII
 styI haeIII
 ncoI asuI hinfI nlaIII bsmal foki
 1822 CAT CTT GCC ATG GCC CCG AAT CAT GCC GTG GTG TCT CGG ATG GAT AAG
 590 His Leu Ala Met Ala Pro Asn His Ala Val Val Ser Arg Met Asp Lys

fnu4HI
 ecoNI alwNI bbvI
 1870 GTG GAA CGC CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG
 606 Val Glu Arg Leu Lys Gln Val Leu Leu His Gln Gln Ala Lys Phe Gly

sau3AI
 mboI mspI
 dpnI hpaII
 xhoII scrFI
 bstYI nciI
 alwI cauII bsrI
 1919 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA
 622 Arg Asn Gly Ser Asp Cys Pro Asp Lys Phe Cys Leu Phe Gln Ser Glu

FIG.-1F

haeIII
 haeI
 eaeI styl
 ddel cfrI pleI ncol
 draIII baI hinfI
 1966 ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG GCC AGA CTC
 638 Thr Lys Asn Leu Leu Phe Asn Asp Asn Thr Glu Cys Leu Ala Arg Leu

sau96I
 auaI
 asuI
 nlaIII ndeI sspI nlaIV
 2014 CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA
 654 His Gly Lys Thr Thr Tyr Glu Lys Tyr Leu Gly Pro Gln Tyr Val Ala

scrFI
 ecoRII
 bstNI
 hgiAI
 bsp1286 mnlI mnlI
 2062 GGC ATT ACT AAT CGT AAA AAG TGC TCA ACC TCC CCC CTC CTG GAA GCC
 670 Gly Ile Thr Asn Leu Lys Lys Cys Ser Thr Ser Pro Leu Leu Glu Ala

ddel
 mstII
 mnlI sau96I
 eco8II mbolI haeIII
 ecoRI bsu36I mbolI asuI aluI
 2110 TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGCCCAG CTCCCCAAGA
 685 Cys Glu Phe Leu Arg Lys DC*

styl
 haeIII
 sau96I
 mbolI scrFI asuI
 ddel earI ecoRII nlaIV
 mnlI aluI bstNI eco109I nlaIV
 2161 AAGCCTCAGC CATTCACTGC CCCAGCTCT TCTCCCAGG TGTGTTGGGG CCTTGGCTCC

ecoNI fokI ddel
 2221 CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA
 2281 AGAAGTAAAA TGAGAAATTT TGTGTGATATT CAAAAAAA

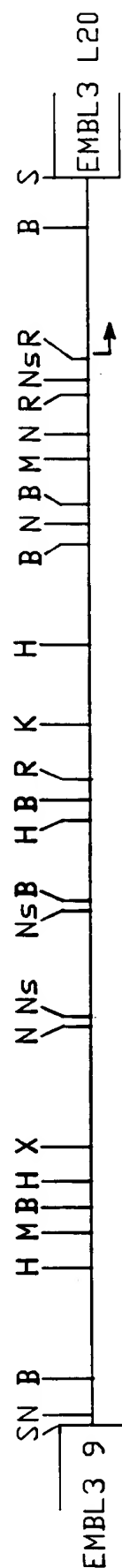
>LENGTH: 2319

FIG.-1G

1435 TGG AGT GGC TTG AGC GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC GCC CTG GTG CTG AAA GGA GAA GCT
362 W S G L S E G S V T C S S A S T T E D C I A L V L K G E A
1522 GAT GCC ATG AGT TTG GAT GGA GGA TAT GTG TAC ACT GCA TGC AAA TGT GGT TTG GTG CCT GTG GCA GAG AAC TAC AAA TCC CAA
391 D A M S L D G G Y V Y T A C K C G L V P V L A E N Y K S Q
1609 CAA AGC AGT GAC CCT GAT CCT AAC TGT GTG GAT AGA CCT GTG GAA GGA TAT CTT GCT GTG GCG GTG GTT AGG AGA TCA GAC ACT AGC
420 Q S S D P D P N C V D R P V E G Y L A V A V R S D T S
1696 CTT ACC TGG AAC TCT GTG AAA GGC AAC AAG TCC TGC CAC ACC GCC GTG GAC AGG ACT GCA GGC TGG AAT ATC CCC ATG GGC CTG CTC
449 L T W N S V K G K S C N T A V D R T A G W N I P M Q L L
1783 TTC AAC CAG ACG GGC TCC TGC AAA TTT GAT GAA TAT TTC AGT CAA AGC TGT GCC CCT GGG TCT GAC CCG AGA TCT AAT CTC TGT GCT
478 F N Q T G S C K F D E Y F S Q S C A P G S D P R S N L C A
1870 CTG TGT ATT GGC GAC GAG CAG GGT GAG AAT AAG TGC GTG CCC AAC AGC AAC GAG AGA TAC TAC GGC TAC ACT GGG GCT TTC CGG TGC
507 L C I G D E Q G E N K C V P N S N E R Y Y G Y T G A F R C
1957 CTG GCT GAG AAT GCT GGA GAC GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT AAC AAT GAG GCA TGG GCT
536 L A E N A G D V A F V K D V T V L Q N T D G N N E A W A
2044 AAG GAT TTG AAC CTG GCA GAC TTT GCG CTG CTG TGC CTC GAT GGC AAA CCG AAG CCT GTG ACT GAC GCT AGA AGC TGC CAT CTT GCC
565 K D L K L A D F A L L C L D G K R K P V T E A R S C H L A
2131 ATG GCC CCG AAT CAT GCC GTG TCT CCG ATG GAT AAG GTG GAA CCG CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG
594 M A P N H A V V S R M D K V E R L K Q V L L H Q Q A K F G
2218 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG
623 R N G S D C P D K F C L F Q S E T K N L L F N D N T E C L
2305 GCC AGA CTC CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA GGC ATT ACT AAT CTG AAA AAG TGC TCA ACC
652 A R L H G K T T Y E K Y L G P Q Y V A G I T N L K K C S T
2392 TCC CCC CTC CTG GAA GCC TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGCCCGAG CTCGCCAAGA AAGCCTCAGC CATTCACCTGC CCCCAGCTCT
681 S P L L E A C E F L R K D
2491 TCTCCCCAGG TGCTGTGGGG CCTTGGCTCC CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA AGAAGTAAAA
2591 TGAGAAATTT TGTGTATTT CAAAAAAA

FIG.-2B

RESTRICTION MAP OF 5' - FLANKING REGION OF α S1 CASEIN GENE



S - SalI

1Kb

B - BclII

R - EcoRI

H - HindIII

M - SmaI

N - NcoI

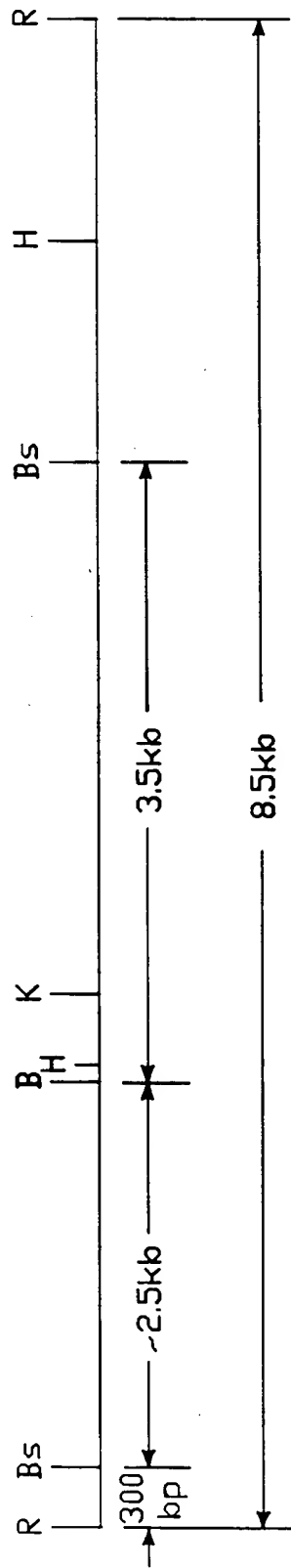
Ns - NsiI

X - XhoI

K - KpnI

FIG.-3

RESTRICTION MAP OF 3' FLANKING
REGION OF α S1 CASEIN GENE



R - EcoRI

Bs - Bst EII

B - BamHI

H - HindIII

K - KpnI

1kb

FIG.-4

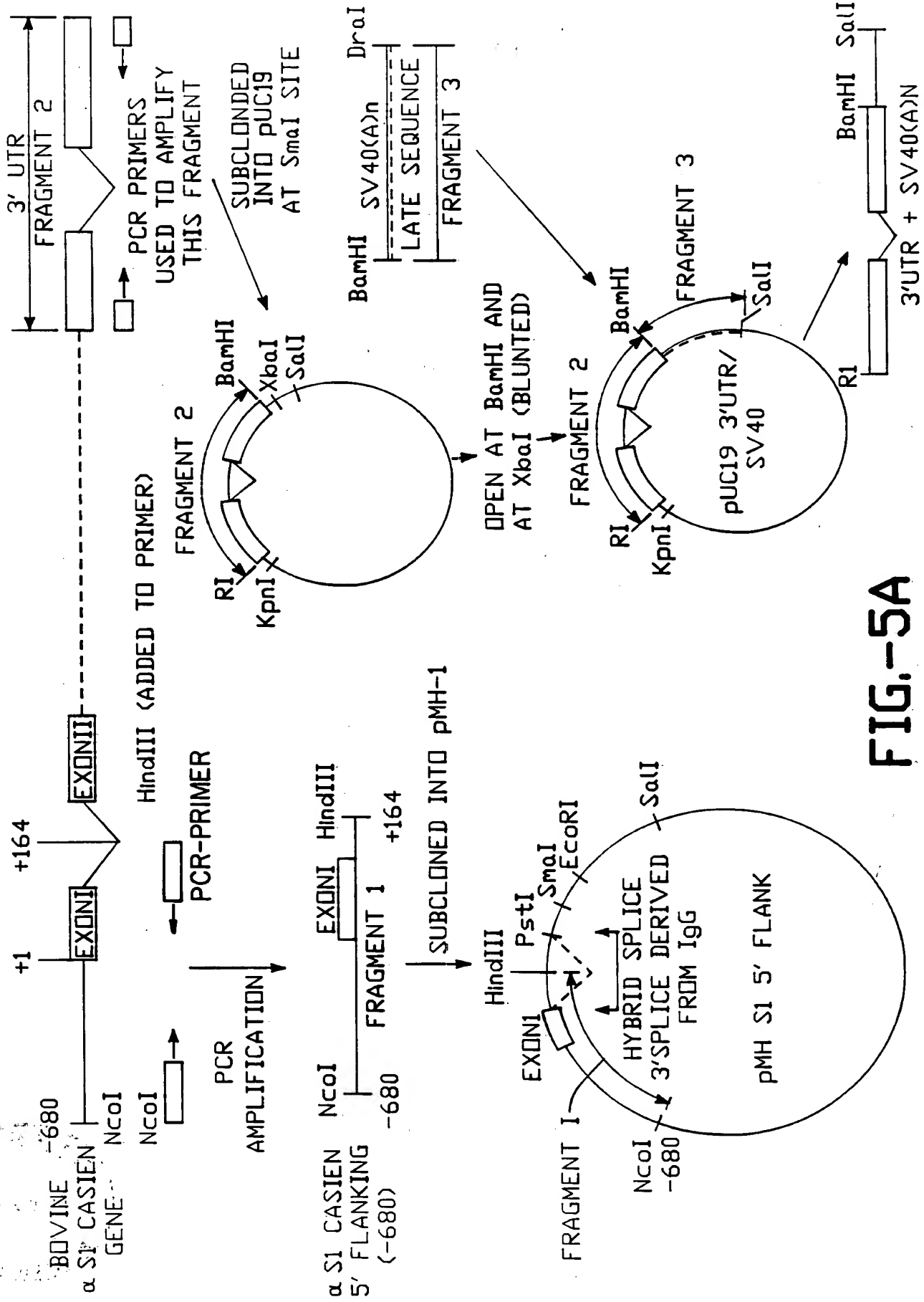


FIG.-5A

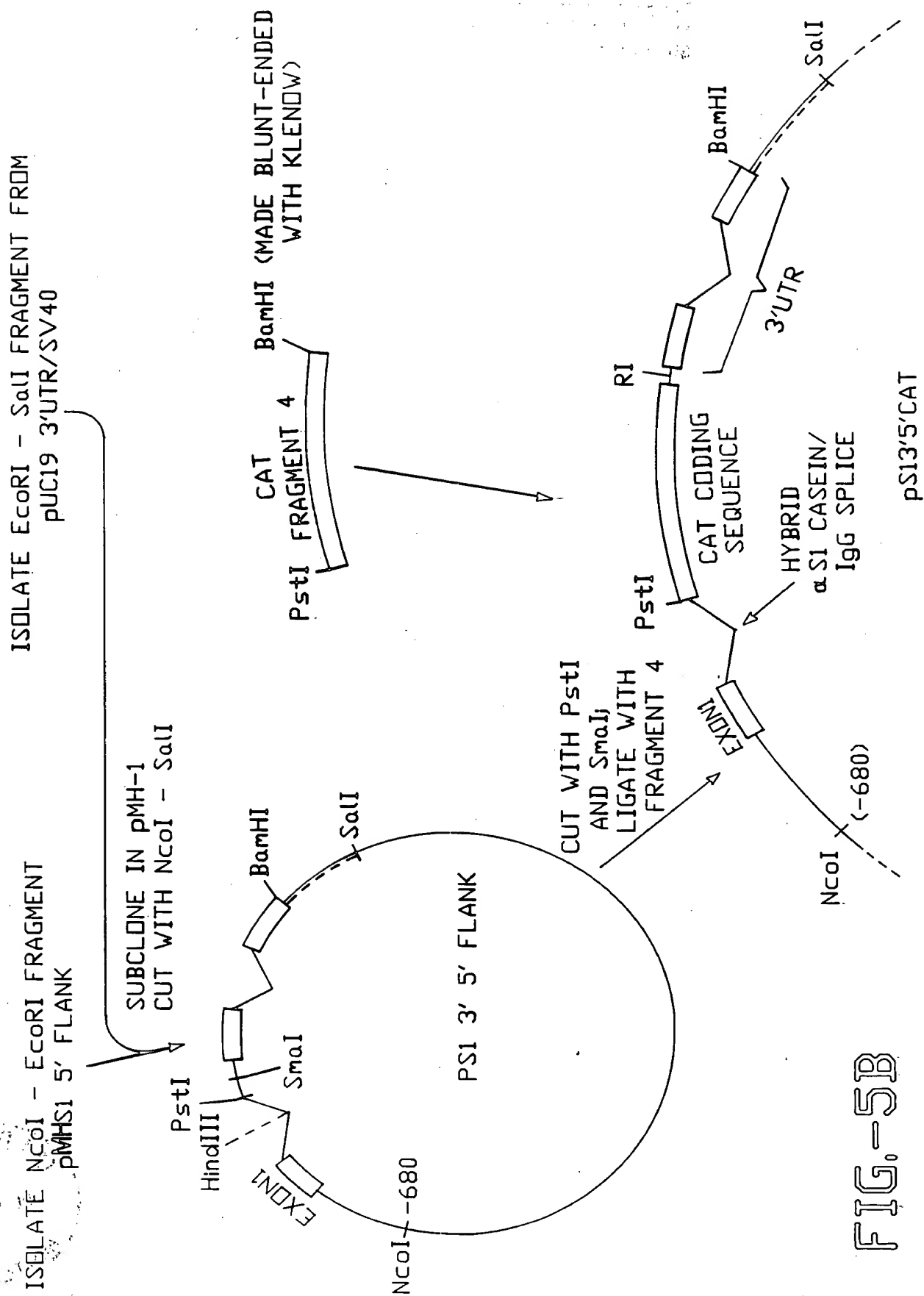


FIG. 5B

ISOLATE CAT + SV40(A)_n LATE SEQUENCE
 PstI SV40 BLUNT
 CAT CODING (A)_n LATE

CUT PstI/SmaI

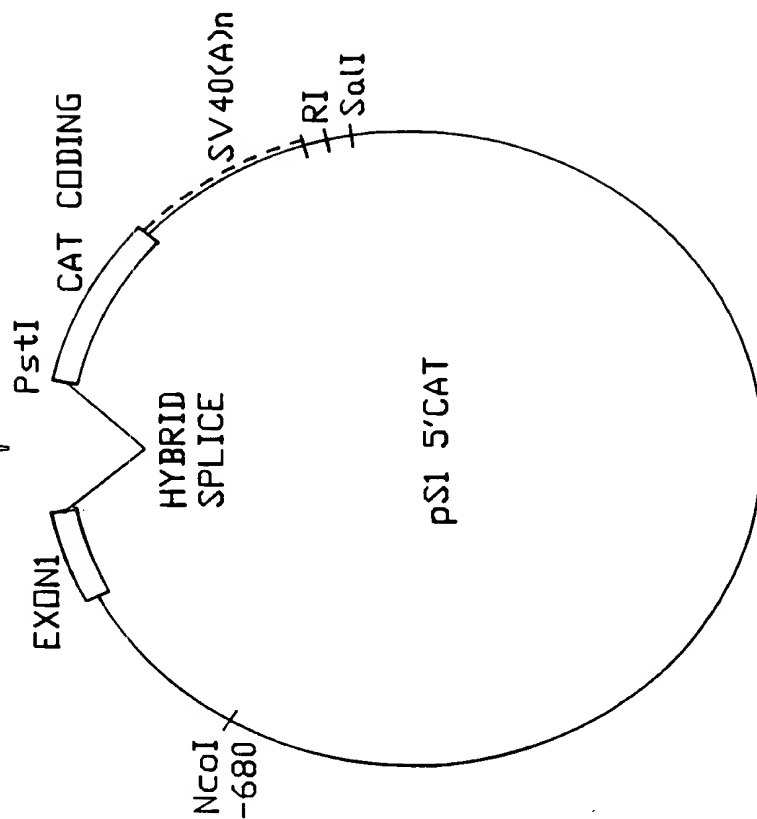
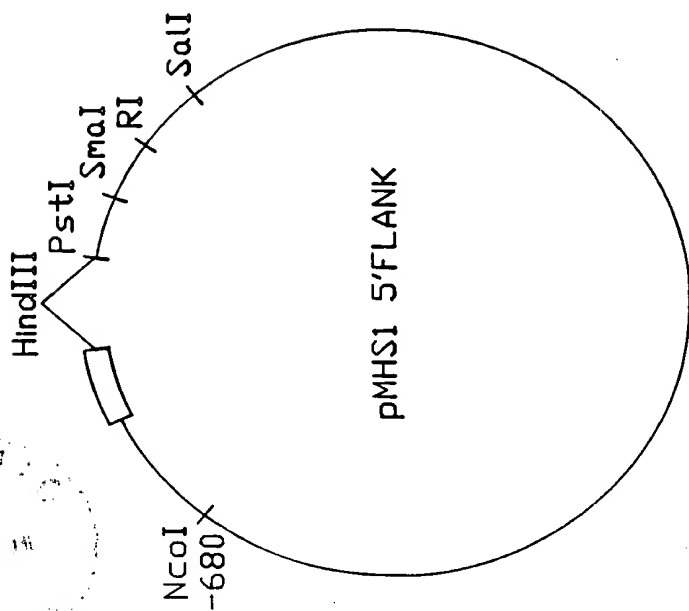


FIG.-5C

NAME pMH-1

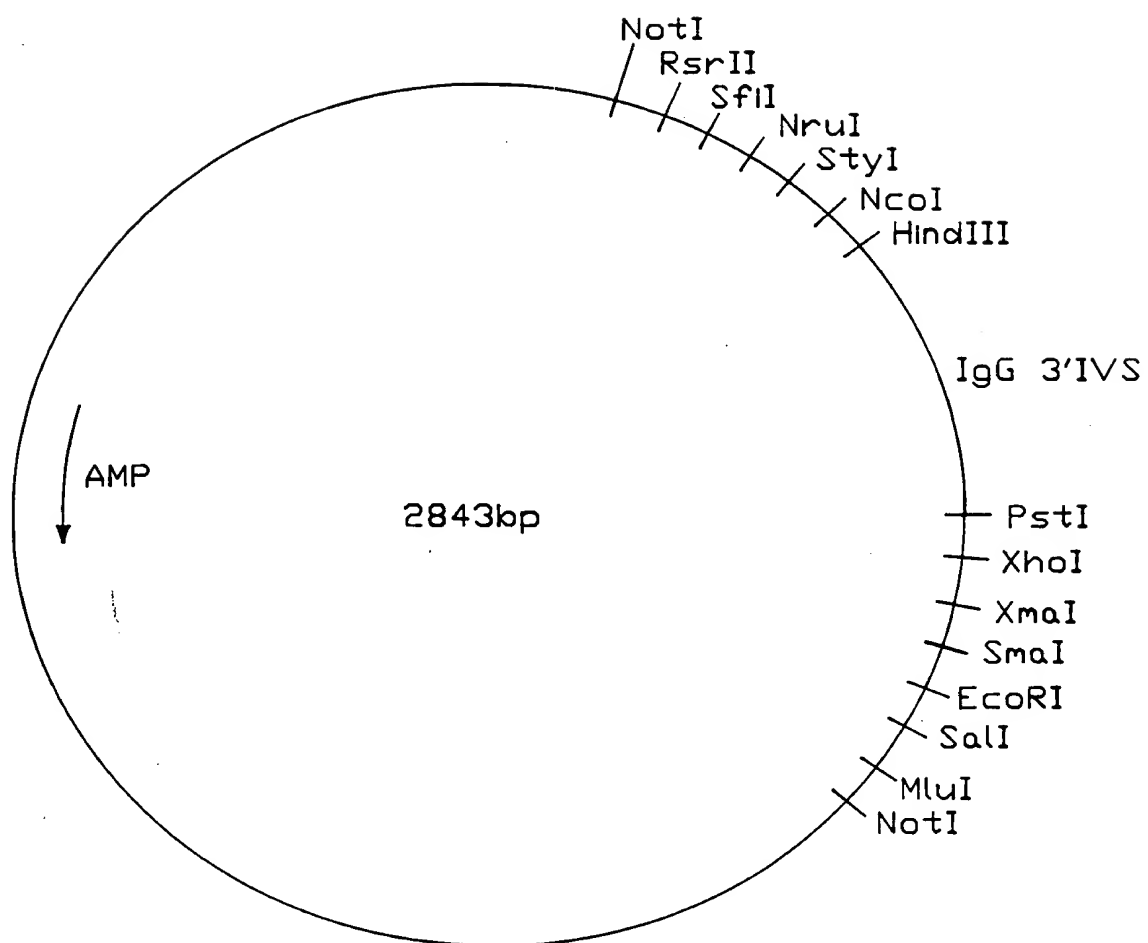
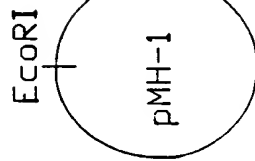
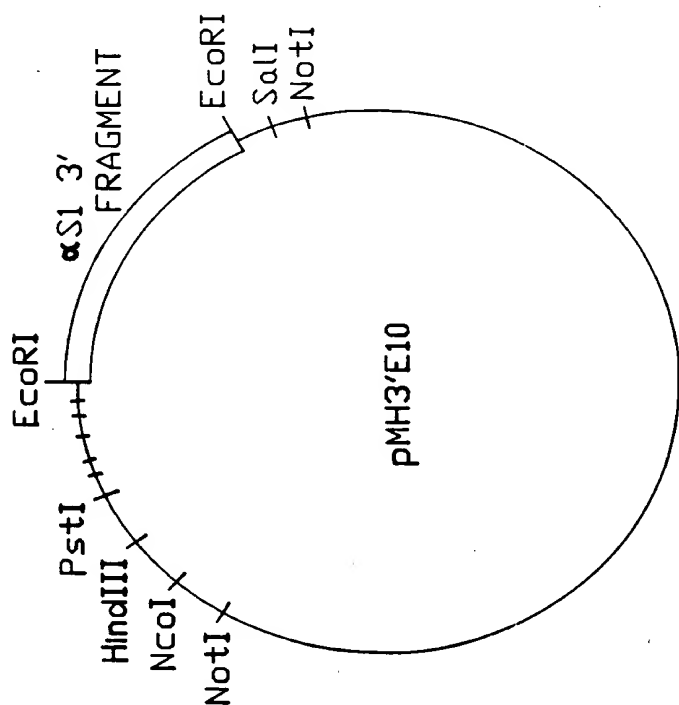


FIG.-6



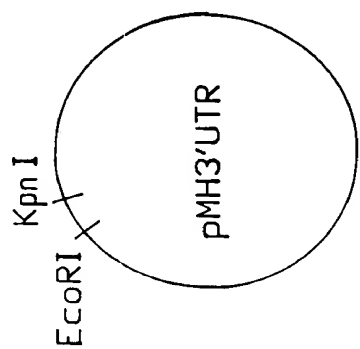
- PLASMID **PMH3'E10** CONTAINS ~ 8.5Kb EcoRI FRAGMENT OF THE 3'-END OF α S1 CASEIN. PMH-1 WAS CUT W/EcoRI + LIGATED TO THIS FRAGMENT



EcoRI
DIGEST

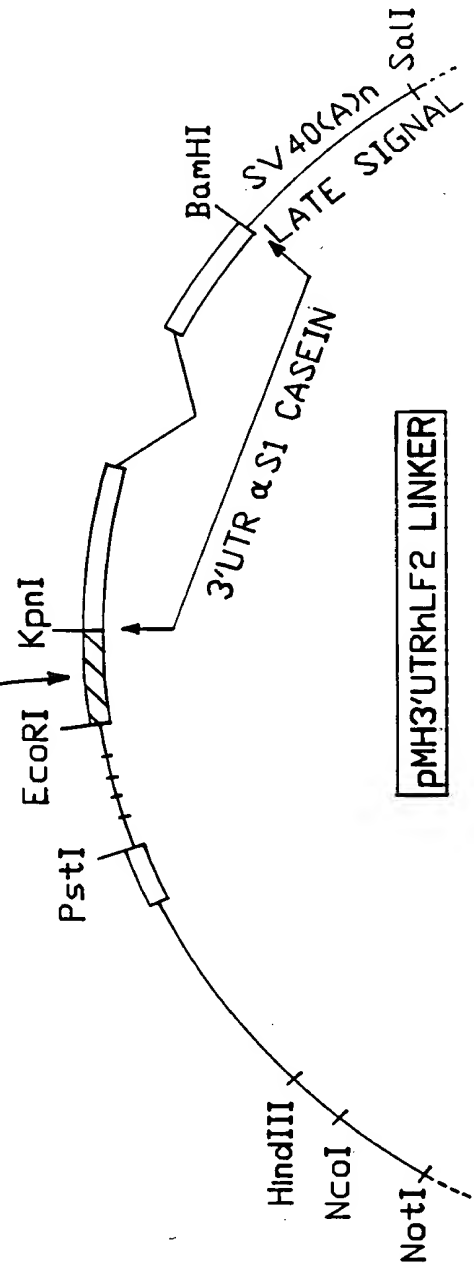
8.5 Kb α S1 CASEIN
+ 3' EcoRI FRAGMENT

- PLASMID **PMH3'UTRhLF2 LINKER**



CUT W/EcoRI
AND KpnI

(FRAGMENT 7)
3' TERMINAL
+ hLF CODING
SEQUENCE
(~ 20bp)



PMH3'UTRhLF2 LINKER

FIG.-7A

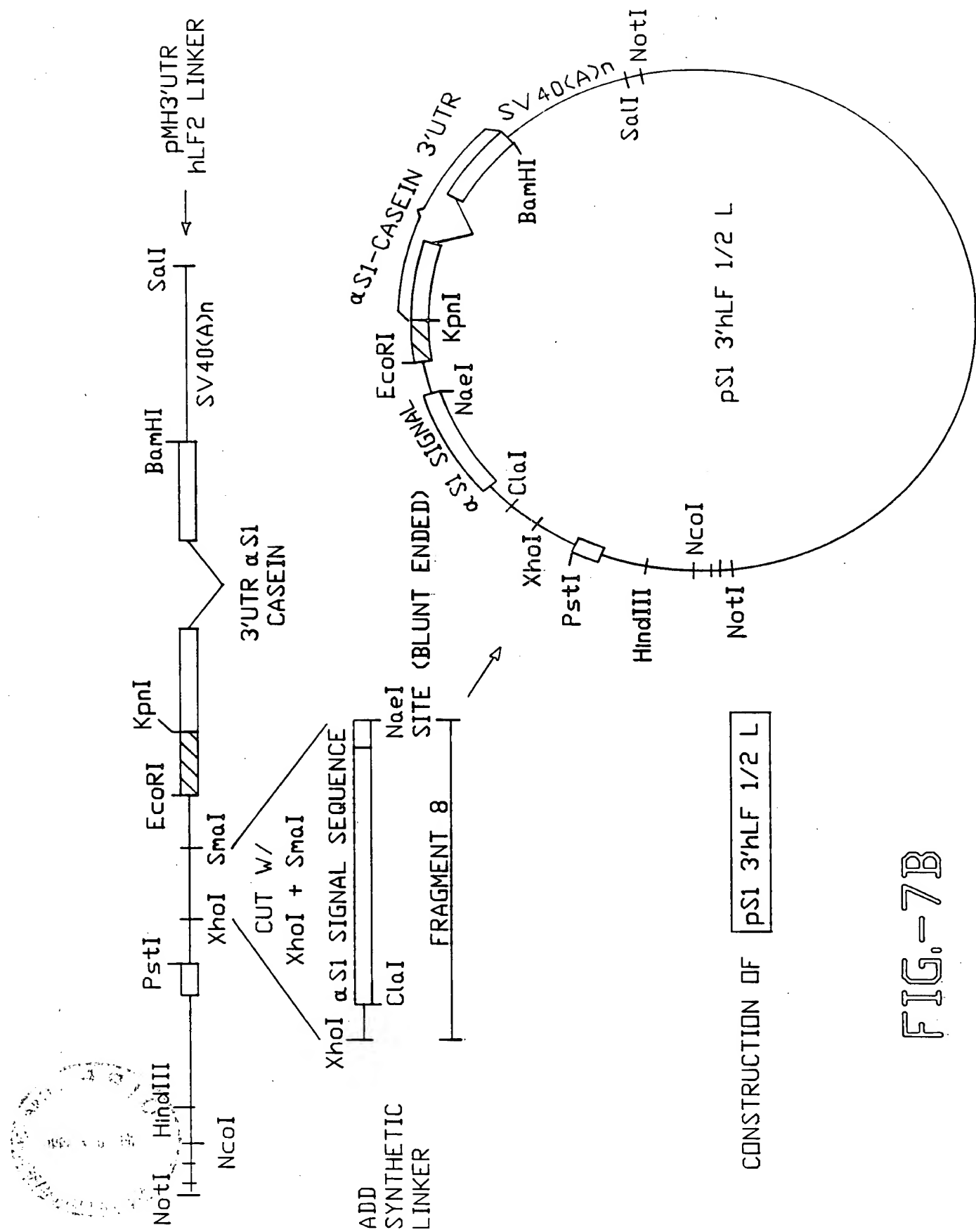


FIG.-7B

CONSTRUCTION OF **pS1 3'UTRhLF**

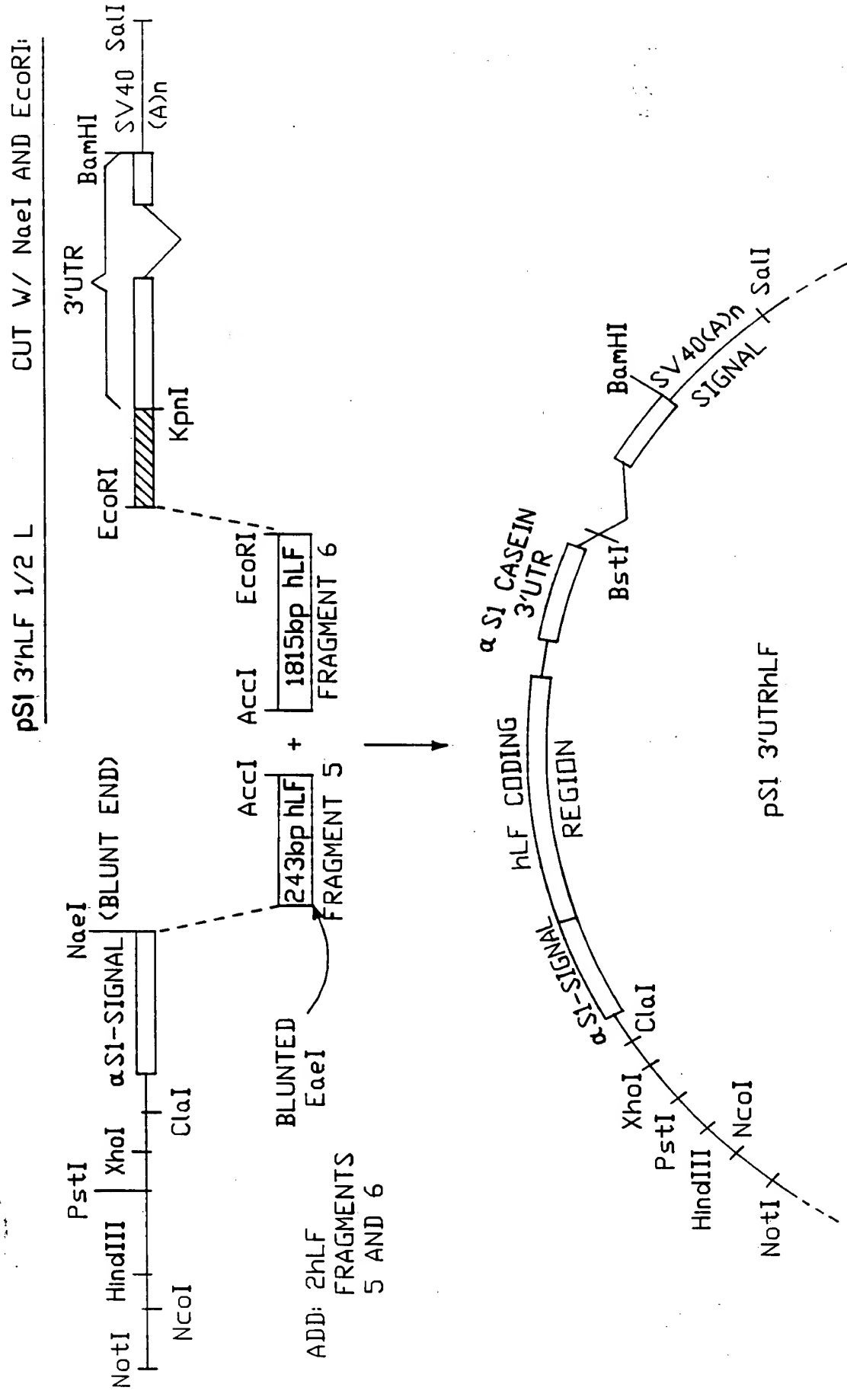


FIG.-7C

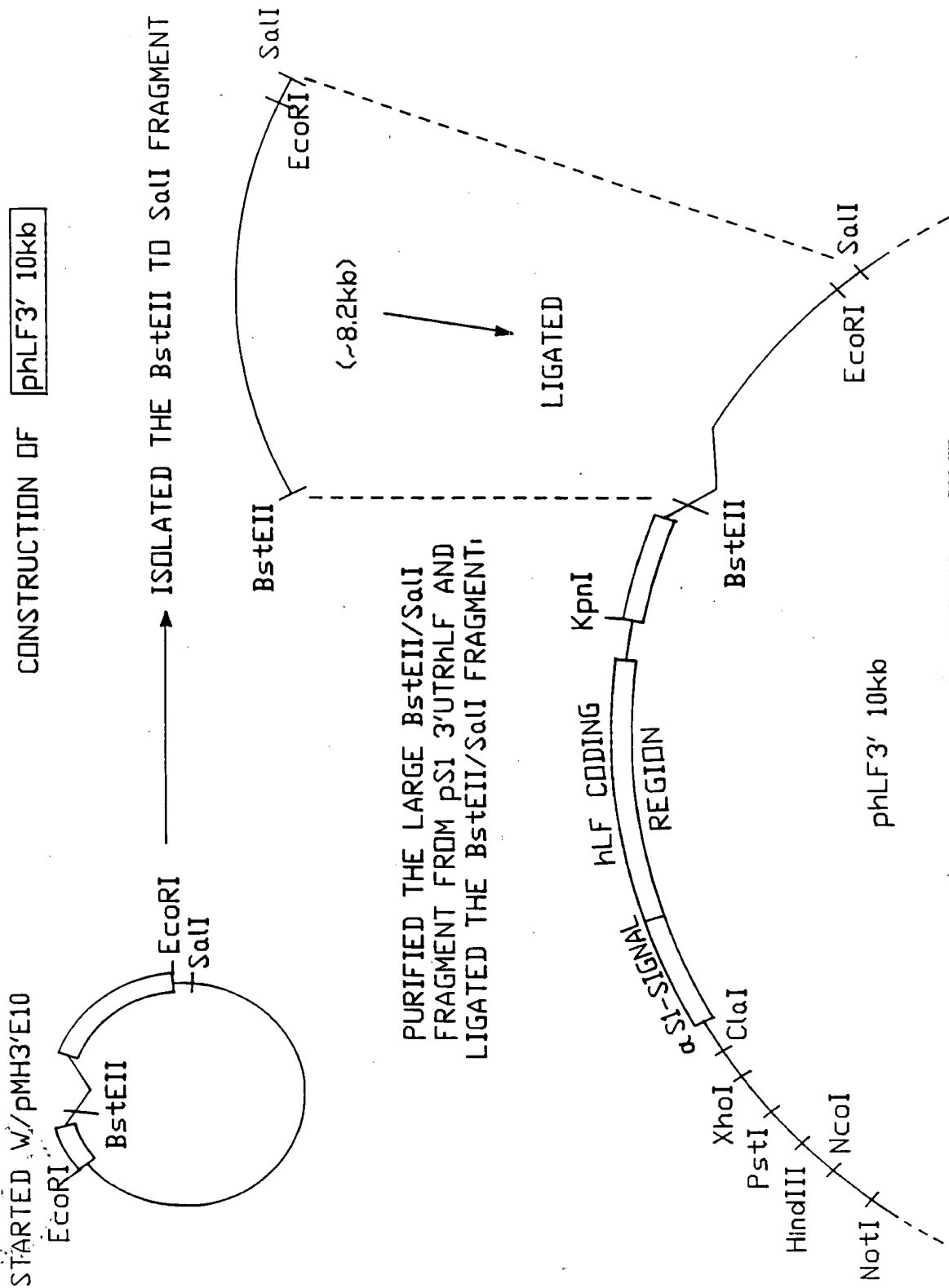
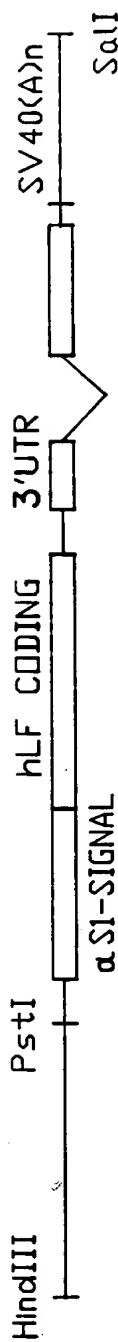


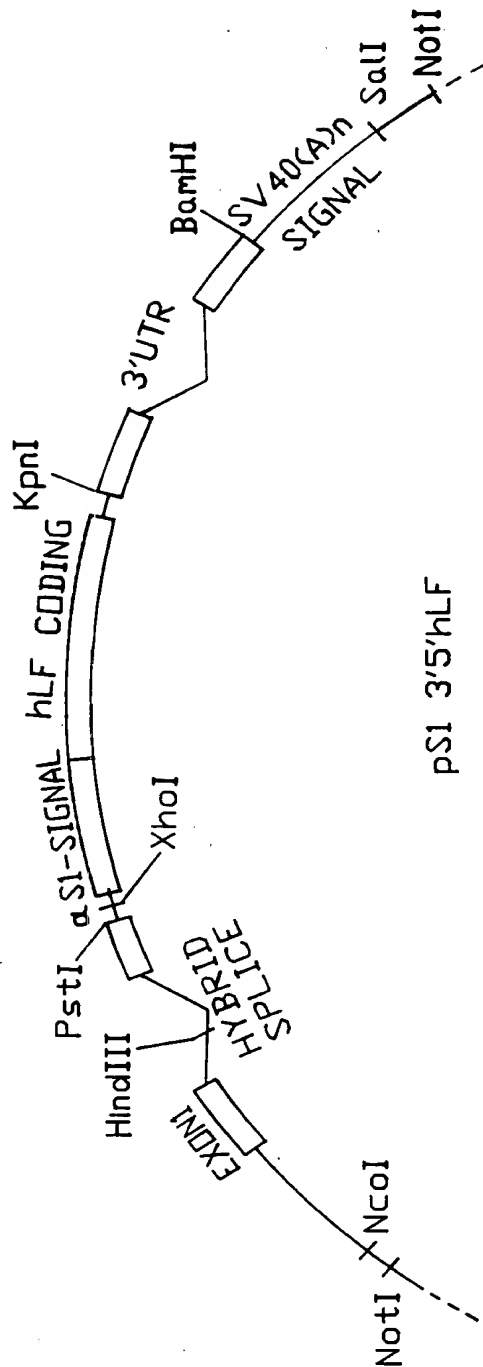
FIG.-7D

CONSTRUCTION OF pS1 3'5'hLF

STARTED. W/pS1 3'UTR hLF. CUT W/HindIII AND SalI AND PURIFIED THIS FRAGMENT CONTAINING THE α S1-CASEIN SIGNAL SEQUENCE, hLF CODING REGION, α S1UTR AND SV40(A)_n.

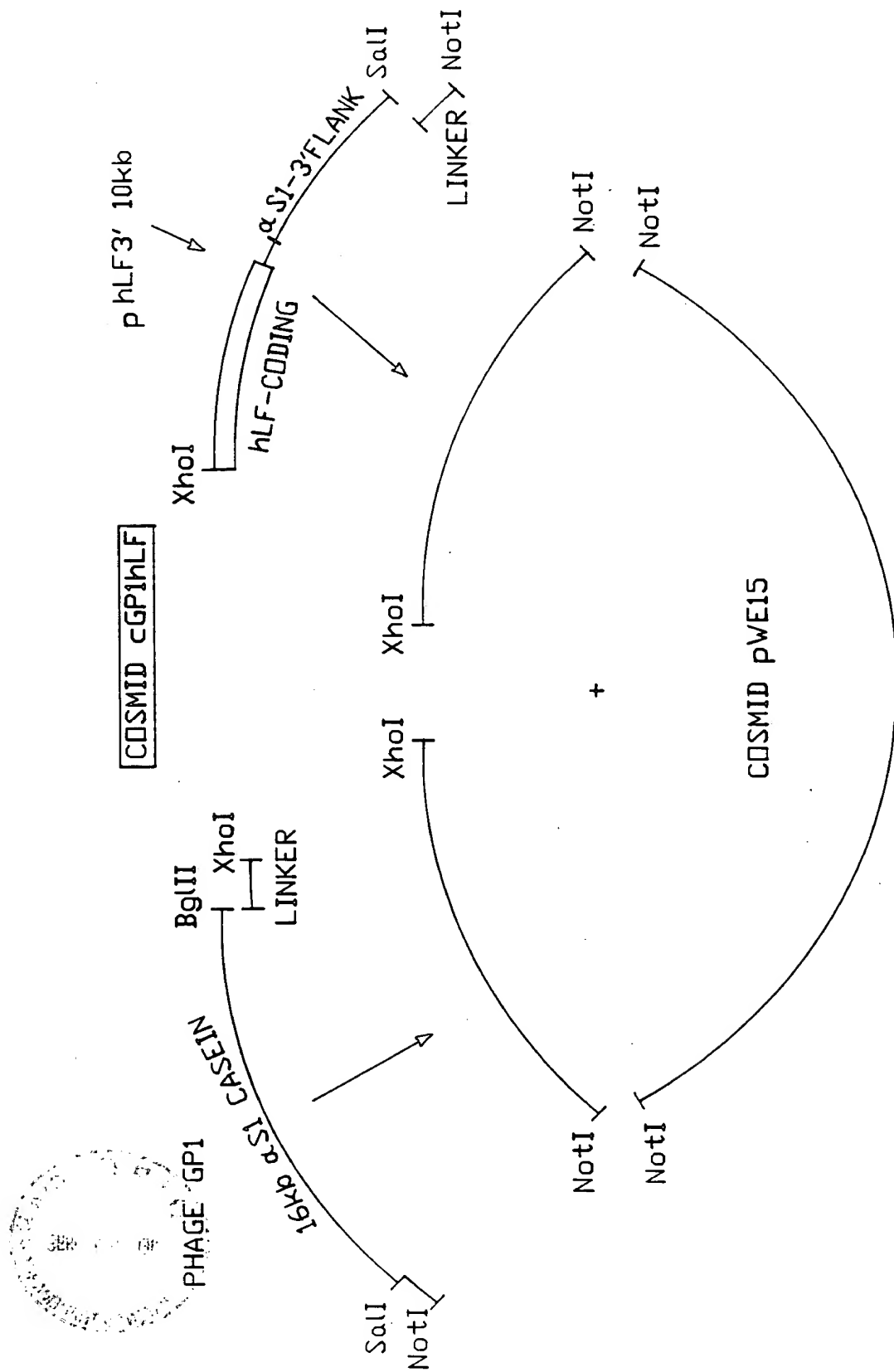


SUBCLONED INTO HindIII/SalI CUT pMHS15' FLANK TO YIELD:



NOTE: pS1 5'hLF WAS MADE BY CUTTING pS13'5'hLF W/KpnI AND BamHI, FOLLOWED BY BLUNTING THE ENDS AND RELIGATING. THIS ELIMINATES THE SPLICED 3'UTR REGION.

FIG.-7E



3 WAY LIGATION. THE DNA FROM THIS COSMID IS PREPARED BY CUTTING WITH *NotI* AND PURIFYING THE EXPRESSION SEQUENCE PRIOR TO MICROINJECTION.

FIG.-7F

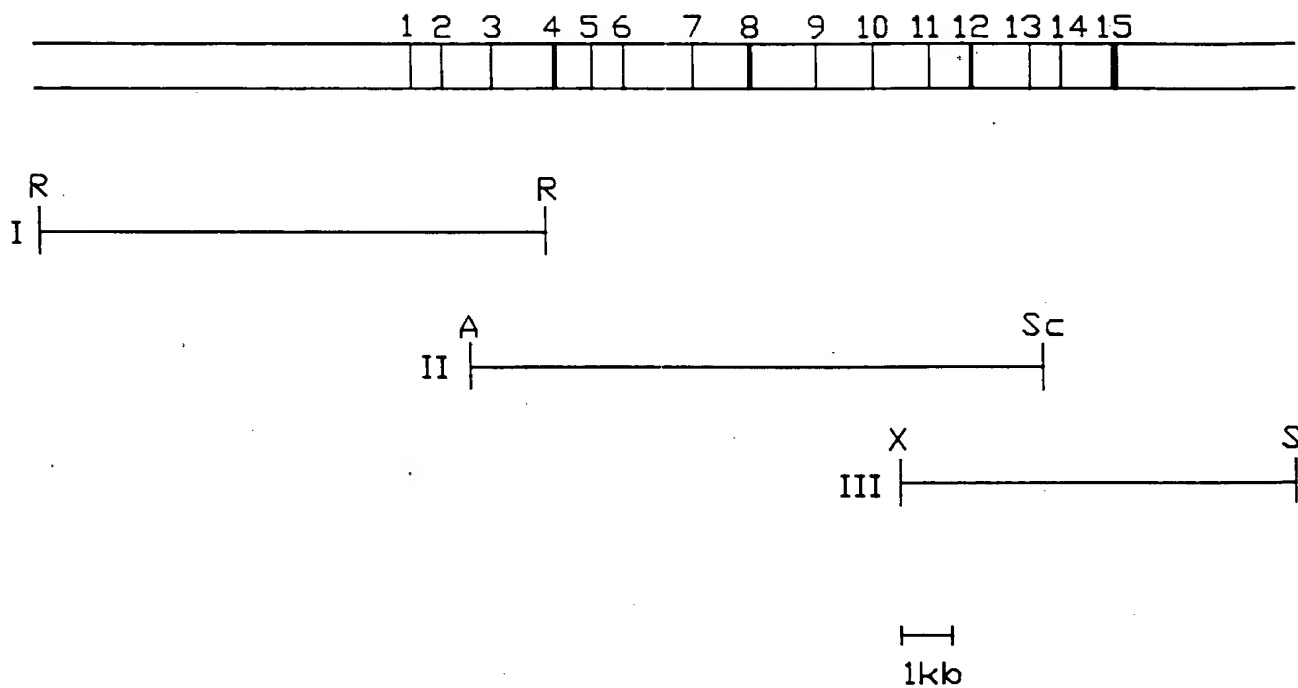


FIG.-8A

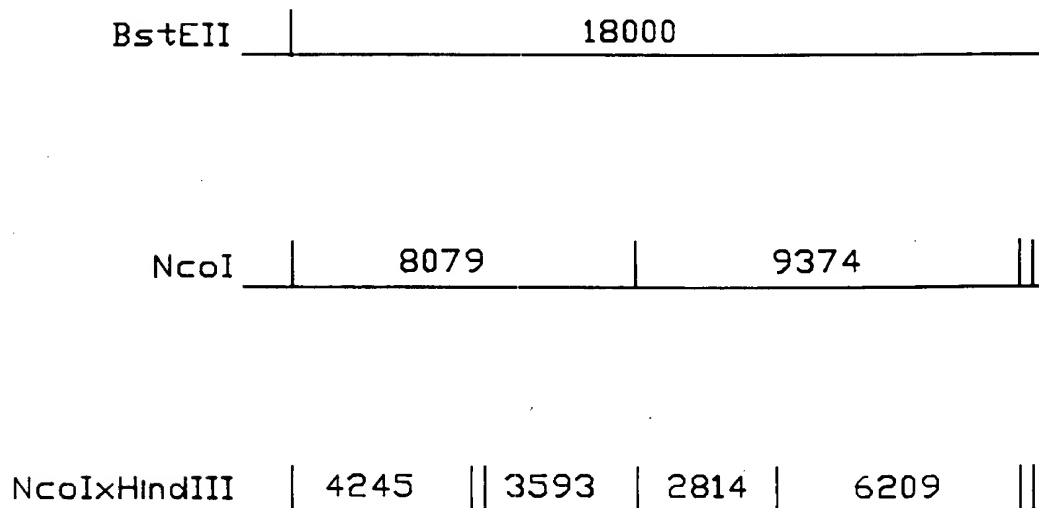


FIG.-8B

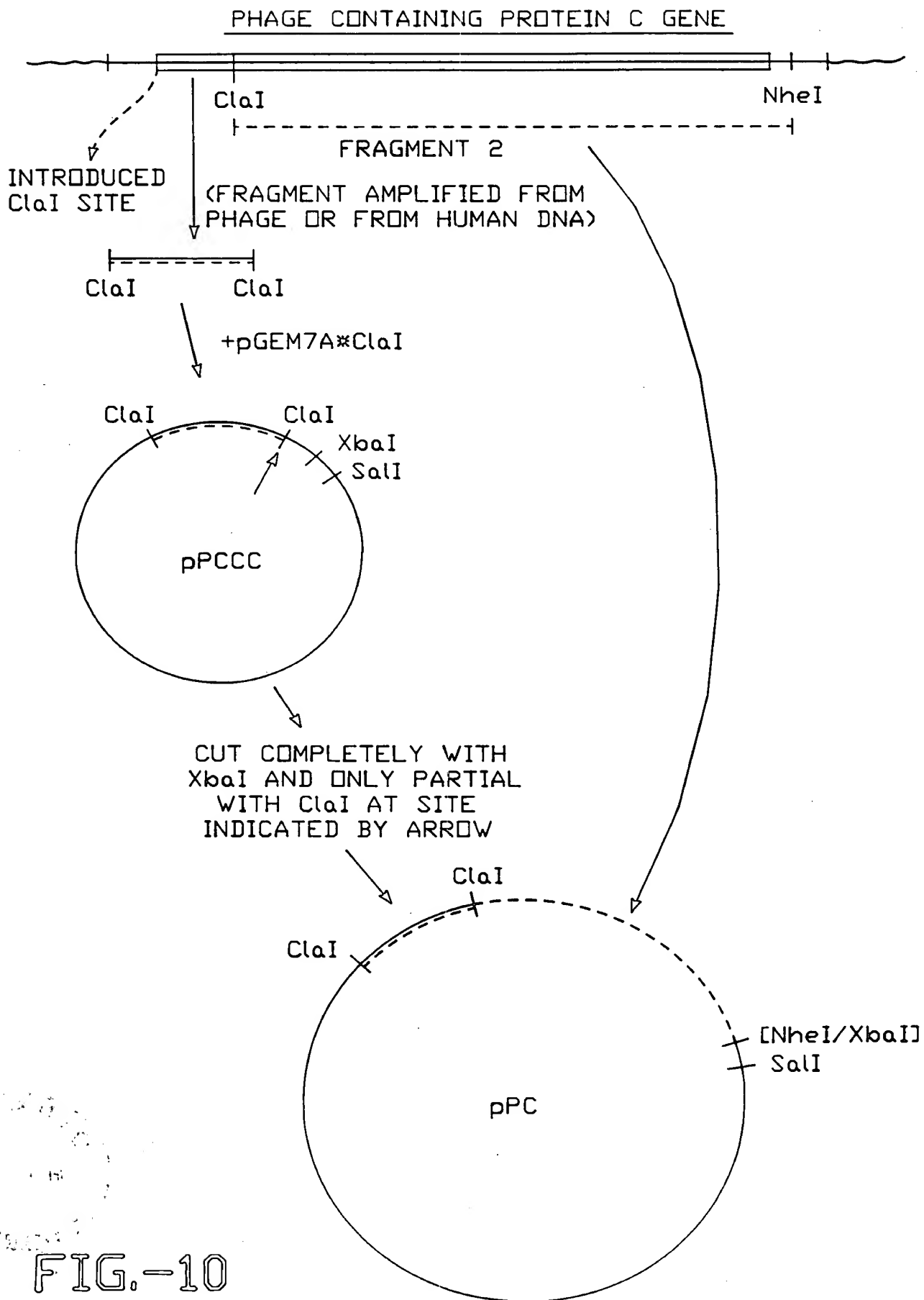
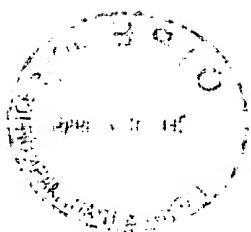


FIG.-10

5'- ATCACCTTGA TCATCAACCC AGCTTGCTGC TTCTTCCCAG
TCTTGGGTTC AAG gtattatgta tacatataac aaaatttcta tgattttcct ctgtctcatc
tttcattctt cactaatagc cagttgtaac ttttctatgt gattgcaagt attggtactt tcctatgata
tactgttagc aagcttgagg tgtggcaggc ttgagatctg gccatacact tgagtgacaa tgacatccac
tttgcccttc tctccacag GTGTCCACTC CCAGGTCCAA CTGCAG -3'

FIG.-11



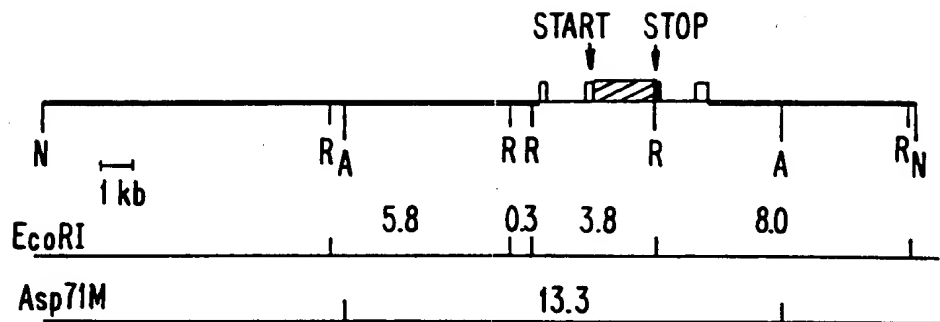


FIG. 12A.

COSMIDS CONTAINING ENTIRE hLF GENE (NOT DRAWN TO SCALE)

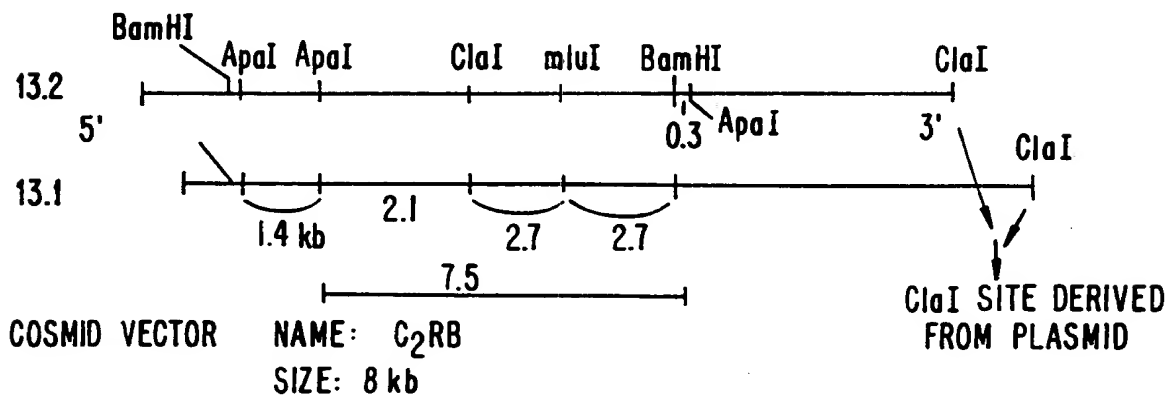


FIG. 13.

9 kb Bam HI hLF FRAGMENT IN pUC19

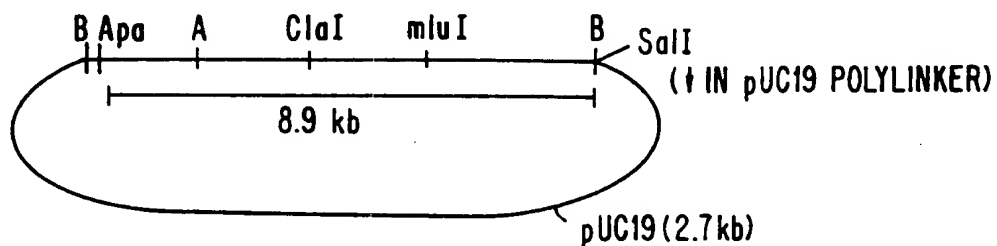


FIG. 14.

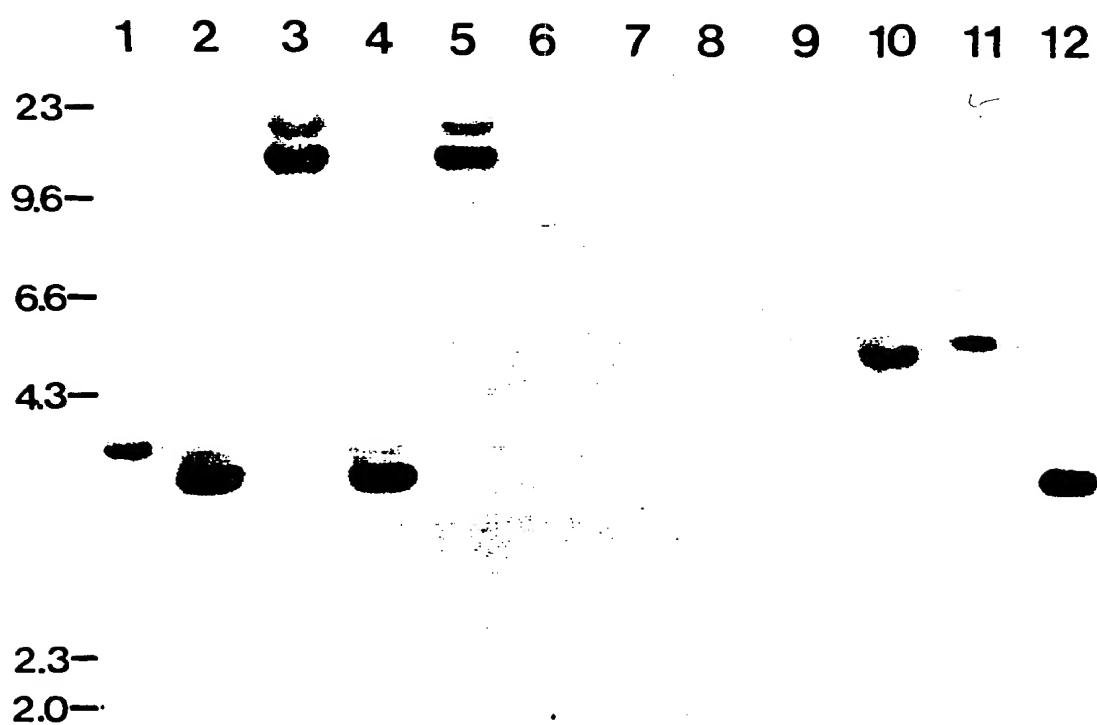
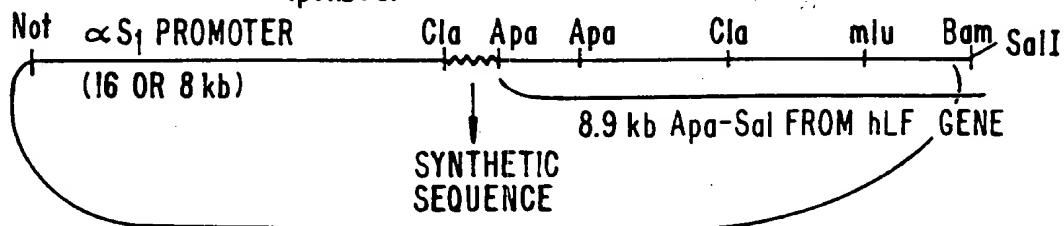


FIG. 12B.



LIGATION PRODUCT OF p16kbCS AND SYNTHETIC SEQ. (Cla-Apa)+ hLF FRAGMENT
(p8kbCS)



CLONING VECTOR: pkUN (4 kb)

CONSTRUCT NAME: 8 hLF gen 9k, OR 16 hLF gen 9k

FIG. 15A.

STRUCTURE OF ClaI-ApaI SYNTHETIC SEQUENCE

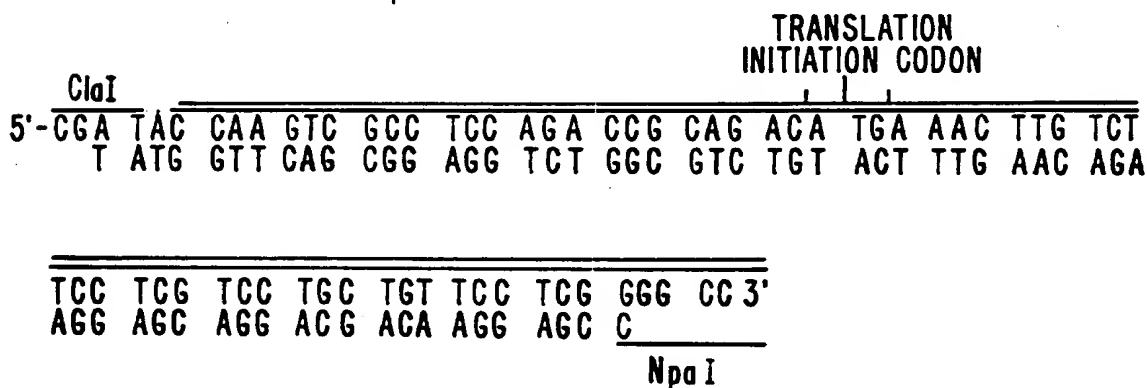
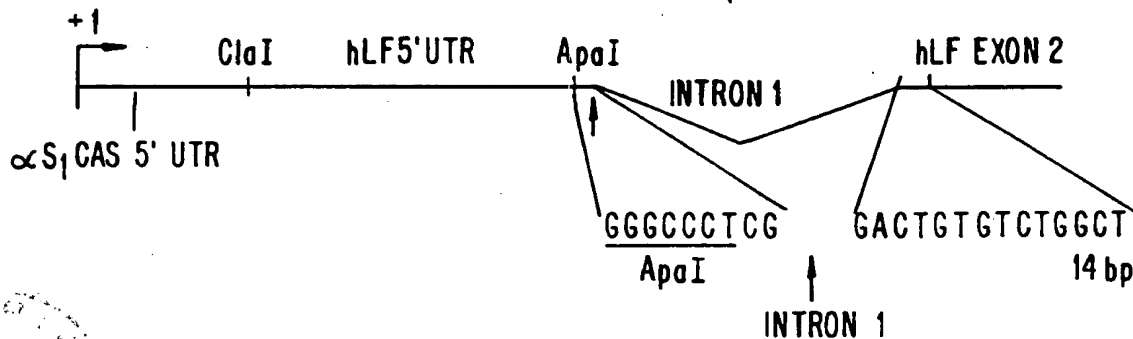


FIG. 15B.

+1: TRANSCRIPTION INITIATION SITE OF BOVINE α S₁-CASEIN GENE



STRUCTURE OF REGION CONTAINING EXON 1 (HYBRID α S₁-CASEIN/hLF EXON) AND PART OF EXON 2 OF THE GENOMIC hLF CONSTRUCTS DEPICTED IN FIGS. 15A THROUGH 17.

FIG. 15C.

COINJECTION

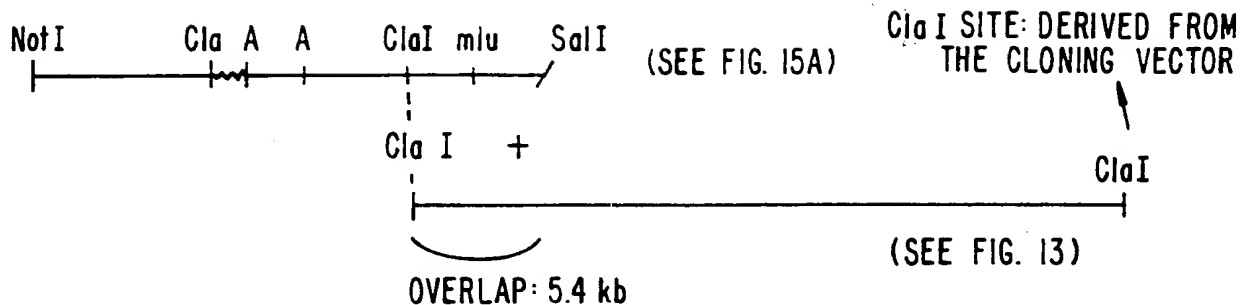
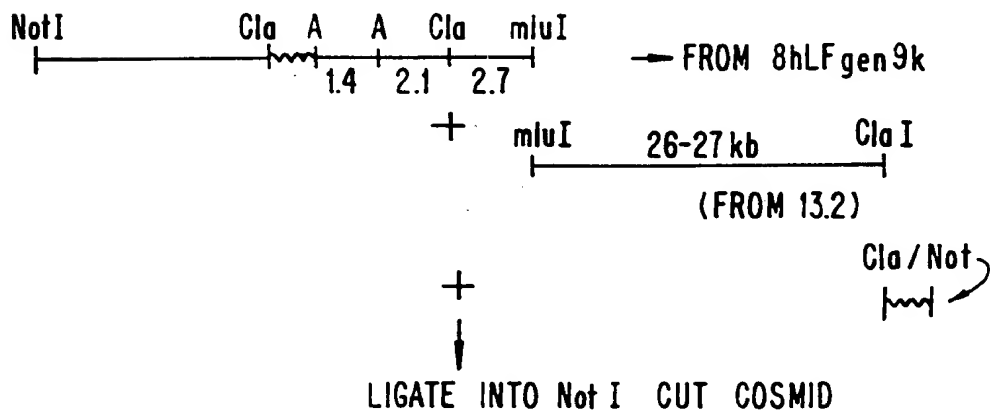


FIG. 16.

GENERATION OF 8hLF GENE



Cla/Not LINKER:

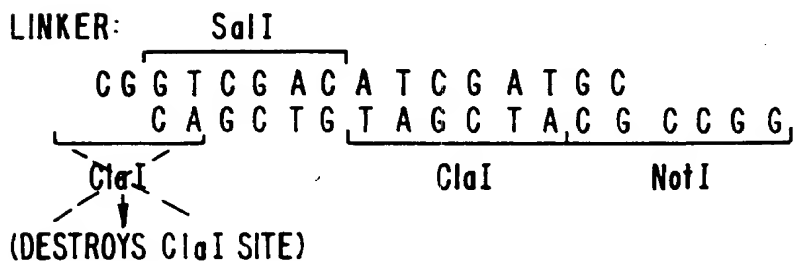


FIG. 17.

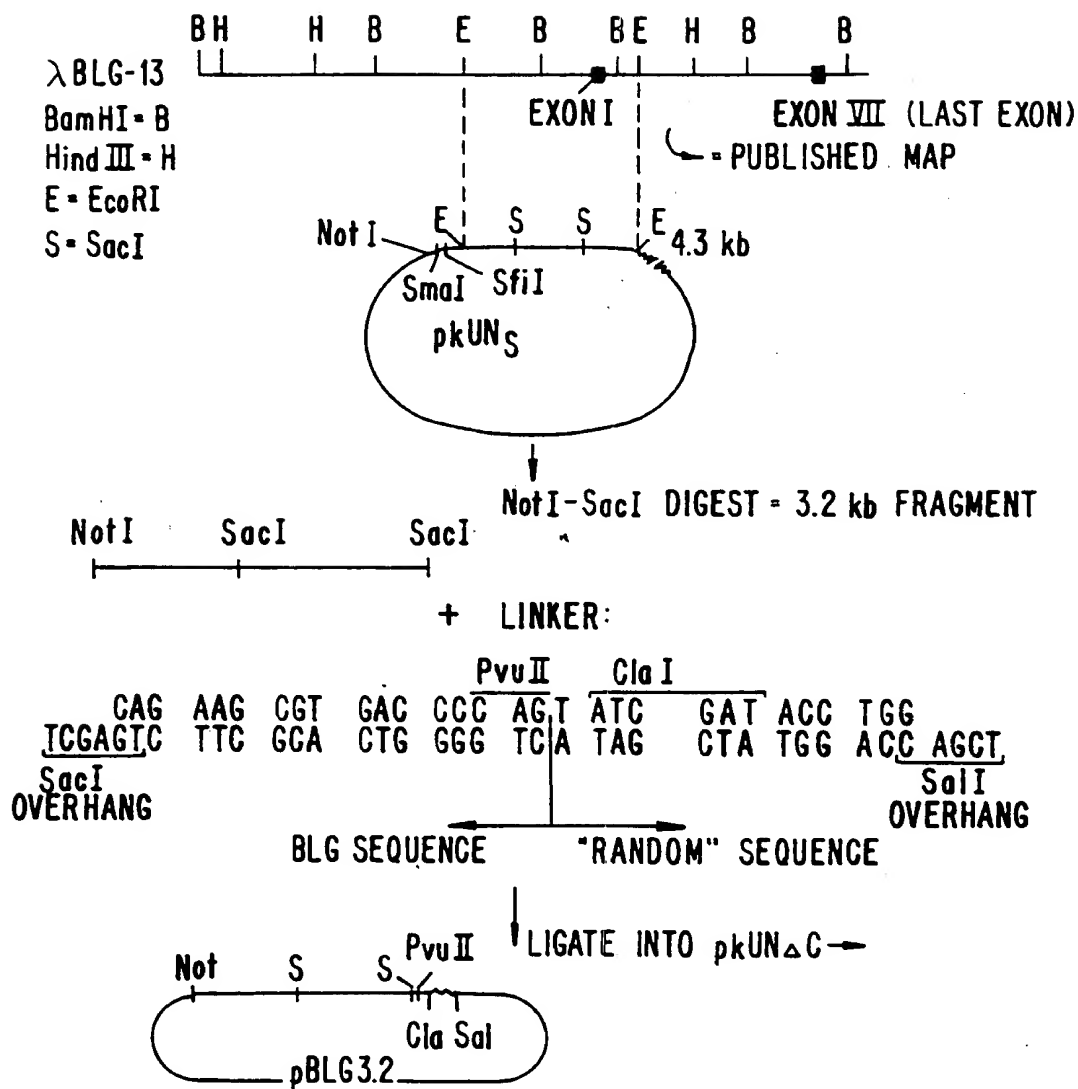


FIG. 18.

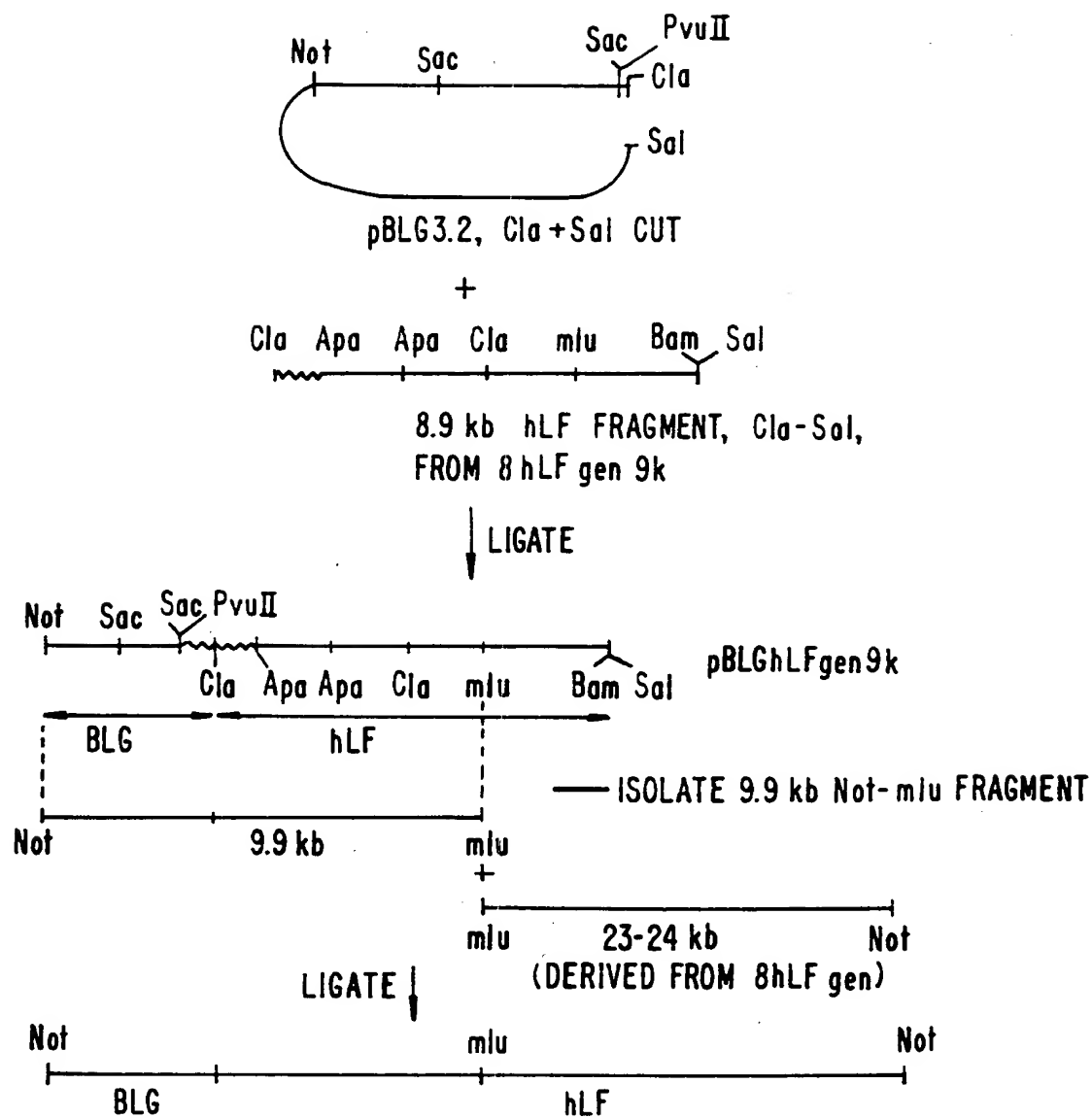


FIG. 19.

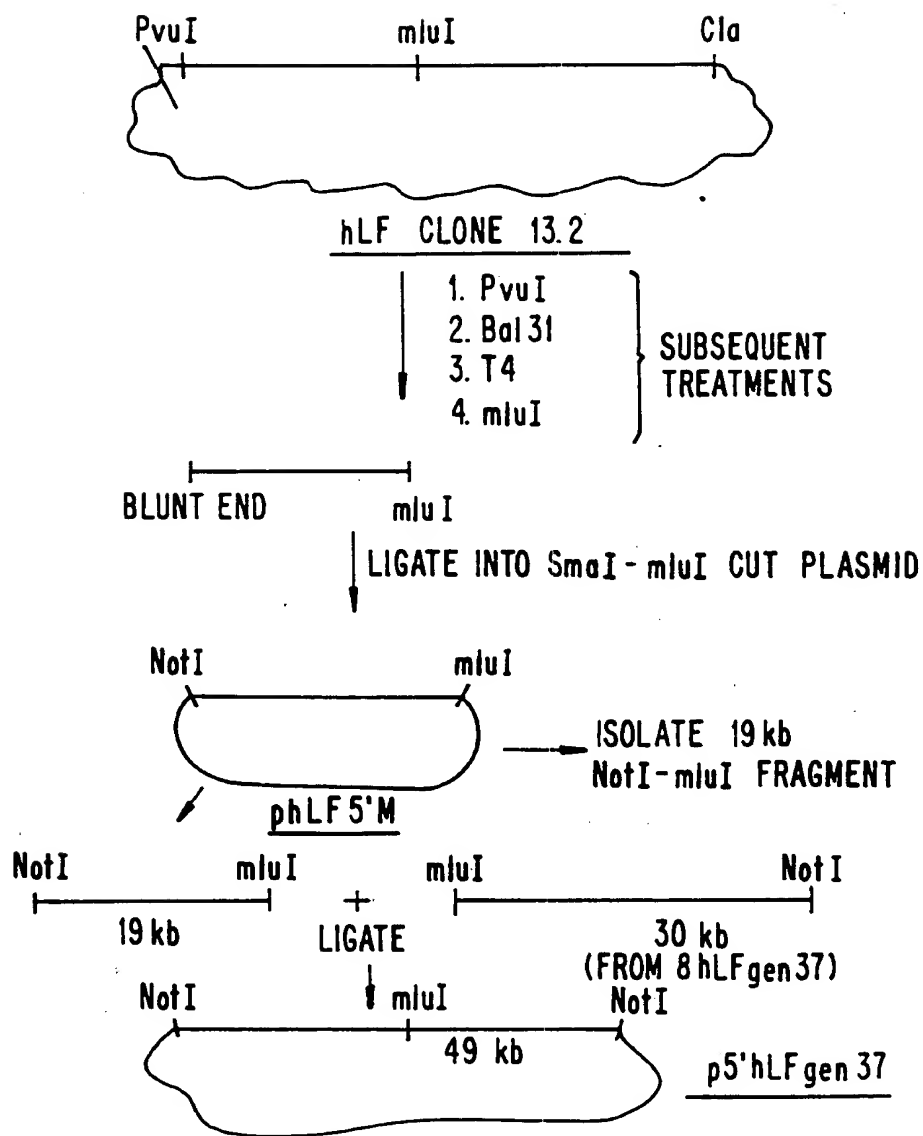


FIG. 20.



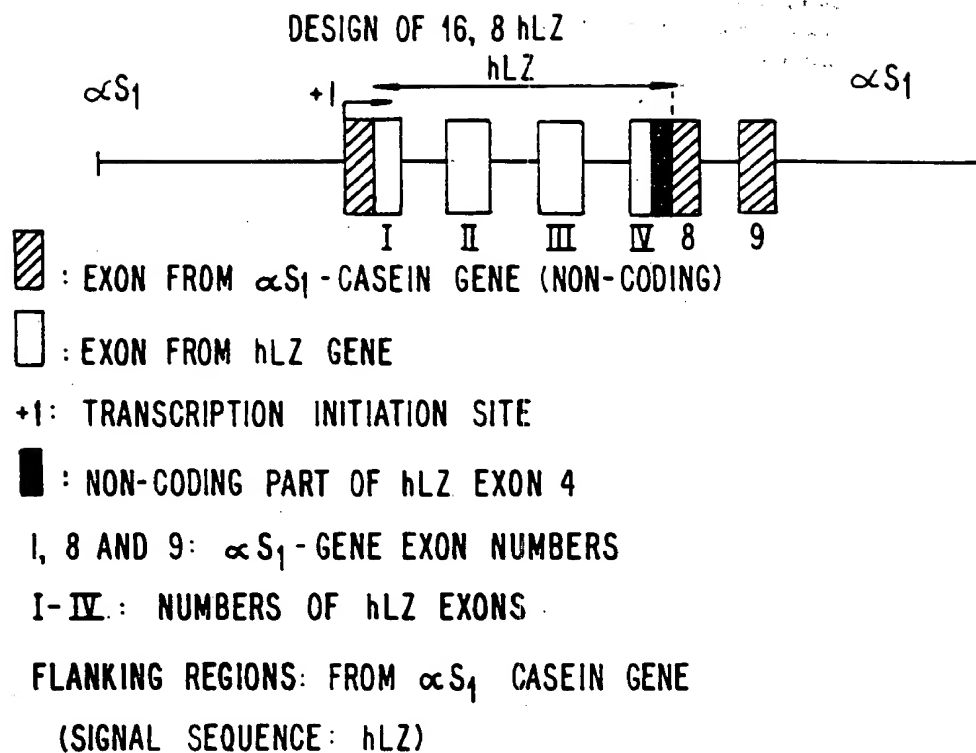


FIG. 21.

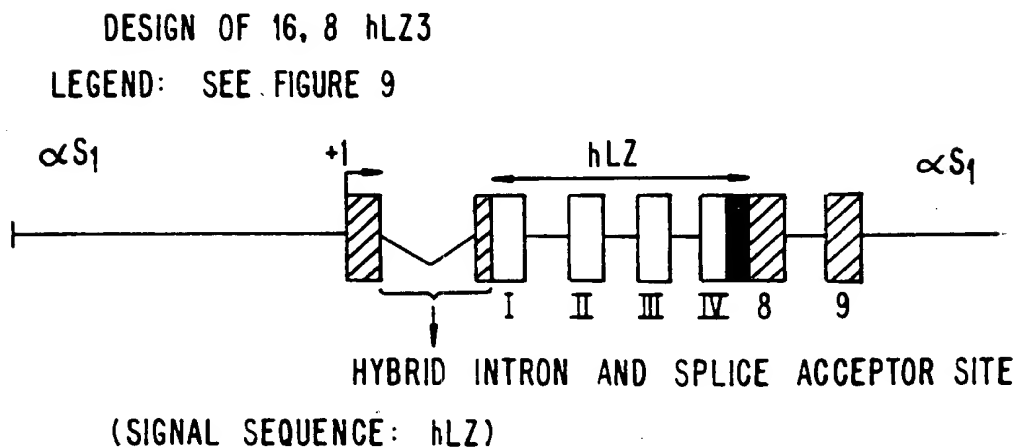


FIG. 22.

Diagram illustrating the structure of the hLZ gene and a 5.3 kb genomic region.

The gene structure shows exons I, II, III, and IV, with a λ -ARM on the left and a λ -ARM on the right. Exon IV is shaded with diagonal lines.

The 5.3 kb region is defined by Hinc II and Sal I sites.

Legend:

- I - IV : hLZ EXON 5
- ▨ : EXON 4, NON-CODING

+3: 5' UTR hLZ
 CAT G G T A G C T A G G G A T C G T G A G A C T G G A T C G T C A G
 KpnI Cla I HincII

Diagram of the pKhLys3'5.3 plasmid. The plasmid is circular and 9.3 kb in size. Restriction sites for ClaI, KpnI, HincII, and SalI are indicated. The hLZ gene is located between the KpnI and SalI sites.

FIG. 23A.

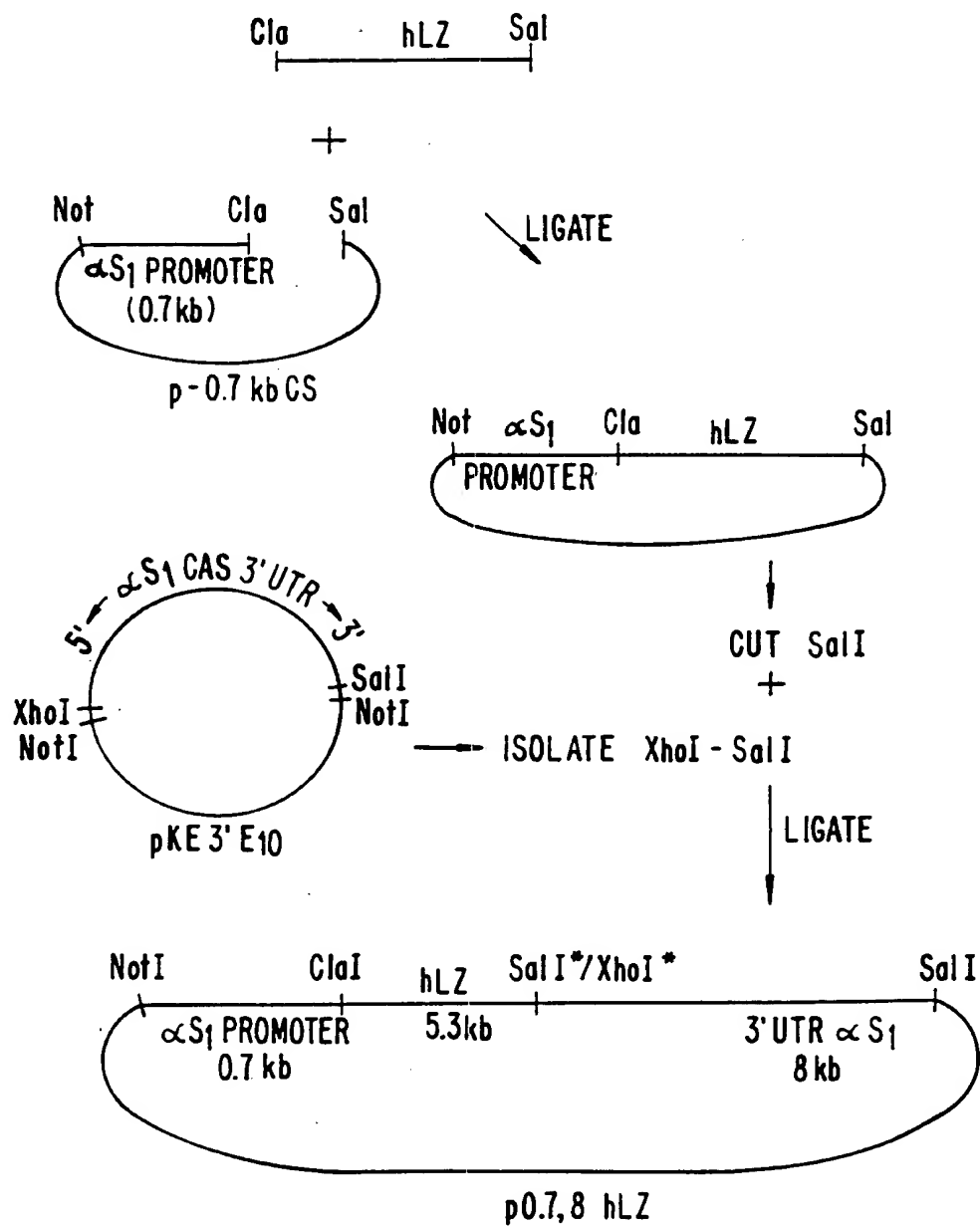


FIG. 23B.



LINKER S₁/S₂:

SalI[⊗] - NotI - SalI[⊗]

⊗: = DESTROYED SITE

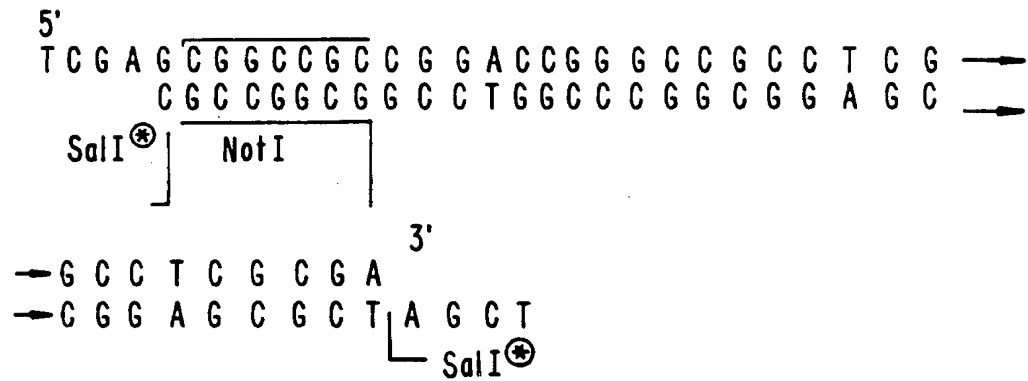


FIG. 23C.

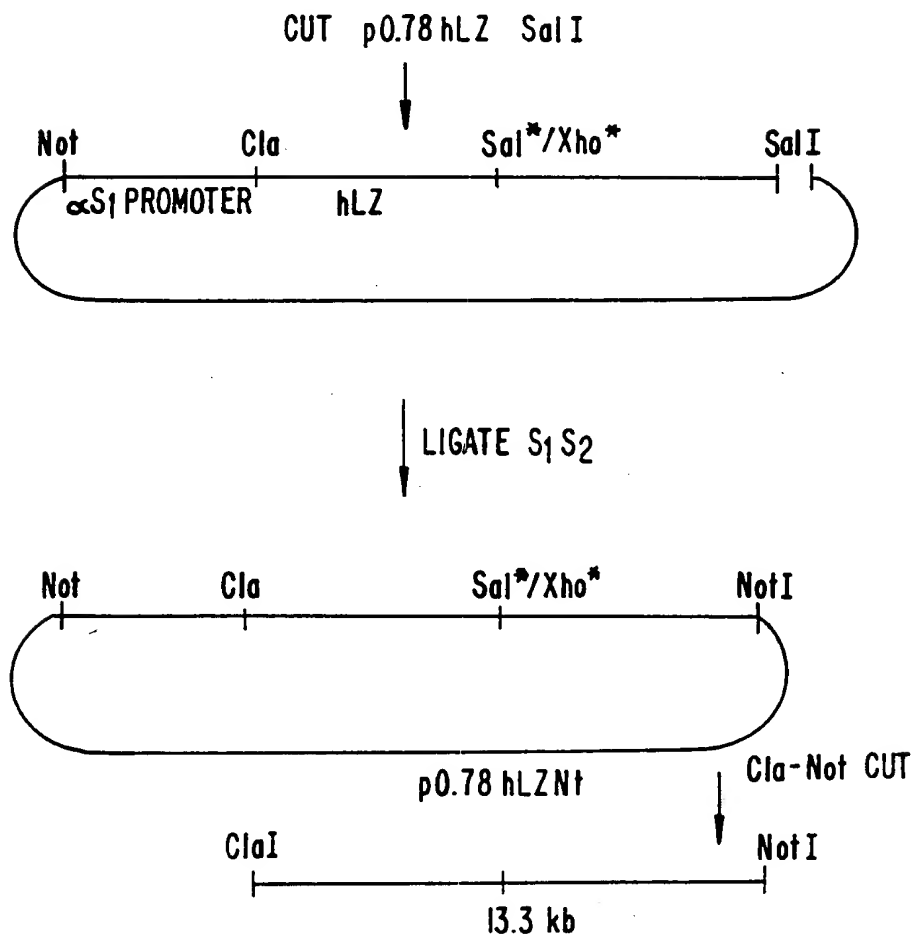


FIG. 23D.

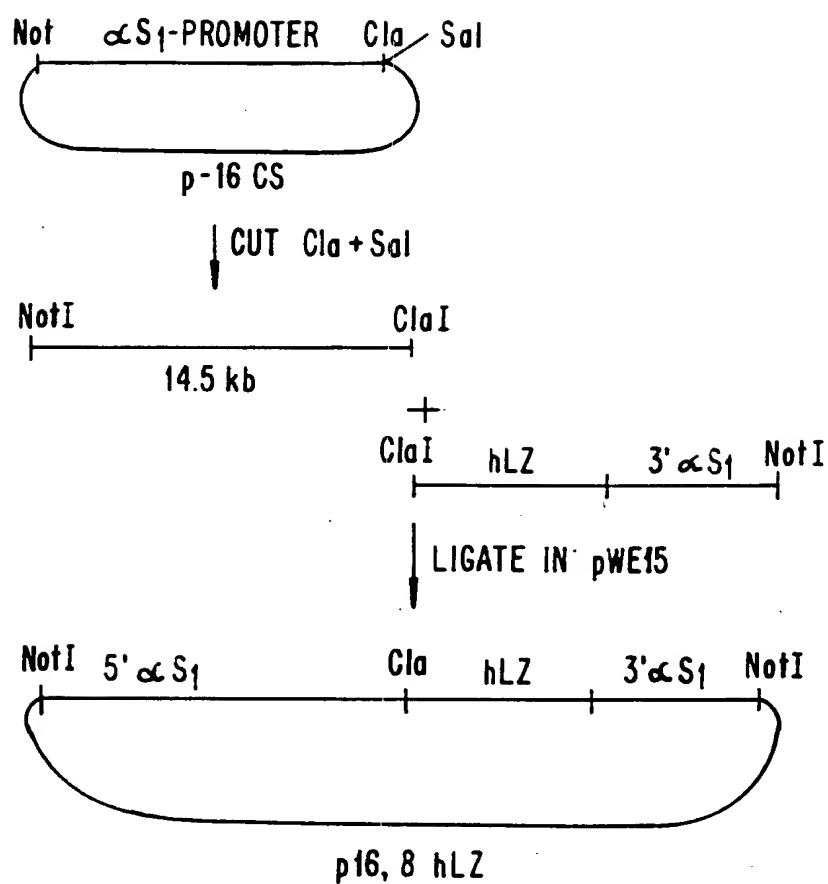


FIG. 23E.



BOVINE 2 GGAAGTGCCTGGAGA...TTAAAATGTGAGAGTGGAGT...GGAGGTTG 44
 SHEEP 84 GGAAGTGTCTCTGGGAGATTTAAAATGTGAGAGGCGGGAGGTGGGAGGTTG 133
 45 GGTCTGTAGGCCTTCCCATCCCACGTGCCTCACGGAGCCCTAGTGCTAC 94
 134 GGCCTGTGGGCTGCCCATCCCACGTGCCTGCATTAGCCCCAGTGCTGC 183
 95 TCAGTCATGCCCCCGCAGCAGGGGTGAGGTCACTTTCCCATCCTGGGGGT 144
 184 TCAGCCGTGCCCCCGCCGAGGGGTGAGGTCACTTTCCCGTCTT.GGGGT 232
 145 TATTATGACTGTTGTCATTGTTGTTGCCATTTTTGCTACCCTAACTGGGC 194
 233 TATTATGACTCTTGTCTATTGCCATTGCCATTTTTGCTACCCTAACTGGGC 282
 195 AGCGGGTGCTTGCAGAGCCCTCGATACTGACCAGGTTCCCCCTCGGAGC 244
 283 AGCAGGTGCTTGCAGAGCCCTCGATAACGACCAGG.TCCTCCCTCGGAGC 331
 245 TCGACCTGAACCCCATGTCACCCTCGCCCCAGCCTGCAGAGGGTGGGTGA 294
 332 TCGACCTGAACCCCATGTCACCCTTCCCCCAGCCTGCAGAGGGTGGGTGA 381
 295 CTGCAGAGATCCCTTTACCCAAGGCCACAGTCAATGGTTTGGAGGAGAT 344
 382 CTGCAGAGATCCCTTCACCCAAGGCCACGGTCAATGGTTTGGAGGAGCT 431
 345 GGTGCCCAAGGCAGAAAGCCACCCTCCA.GACACACCTGCCCCCAGTGCTG 393
 432 GGTGCCCAAGGCAGAGGCCACCCTCCAGGACACACCTGTCCCCAGTGCTG 481
 394 GCTCTGACCTGTCCTTGTCTAAGAGGCTGACCCCAAGAGTGTTCCTGGCG 443
 482 GCTCTGACCTGTCCTTGTCTAAGAGGCTGACCCCGGAAGTGTTCCTGGCA 531
 444 CTGGCAGCCAGCCTGGACCCAGAGCCTGGACACCC.CCTGCGCCCCCACT 492
 532 CTGGCAGCCAGCCTGGACCCAGAGTCCAGACACCCACCTGTGCCCCCGCT 581
 493 TCTGGGGGGGTACCAGGAACCGTCCAGGCCCAGA..GGGCTTCCTGCTT 540
 582 TCTGGGGTC.TACCAGGAACCGTCTAGGCCCAGAGGGGGACTTCCTGCTT 630
 541 GGCTCGAATGGAAGAAGGCCTCCTATTGTCTTTCGTAGAAGGAAGCAACC 590
 631 GGCTTGGATGGAAGAAGGCCTCCTATTGTCC.TCGTAGAGGAAGCCACC 679
 591 CCAAGGGCCCAAGGATAGGCCAGGGGGGATTCCGGGAACCGCGTGCTT.CC 639
 680 CCGGGGCTGAGGATGAGCCAAAGTGGGATTCCGGGAACCGCGTGCTTGG 729
 640 GCGCGGGCCCGGGCTGGCTGGCTGGC..CCTCCTCCTGTATAAGGCCCCG 687
 730 GGGCCAGCCCGGGCTGGCTGGCTGCGCTCCTGTATAAGGCCCCA 779

FIG. 24A.

```
688 AGCCCG. CTGTCTCAGCCCTCCACTCCCTGCAGAGCTCAGAAGCGTGACC 736  
    |||||      |||||      |||||      |||||      |||||      |||||  
780 AGCCTGCCGTGTCTCAGCCCTCCACTCCCTGCAGAGCTCAGAAGCACGACC 829  
          *  
737 CCAGCTGCAGCCATGAAGTGCCTCCTGCTTGC.....CCTGGCCCTCAC 780  
    |||||      |||||      |||||      |||||      |||||      |||||  
830 CCAGCTGCAGCCATGAAGTGCCTCCTGCTTGCCTGGCCCTGGCCCTCGC 879  
  
781 CTGTGGCGCCAGGCCCTCATCGTCAAC 808  
    |||||      |||||      |||||      |||||  
880 CTGTGGCGTCCAGGCCATCATCGTCAAC 907
```

FIG. 24B.

CONSTRUCTION OF 16.8 A hLZ3:

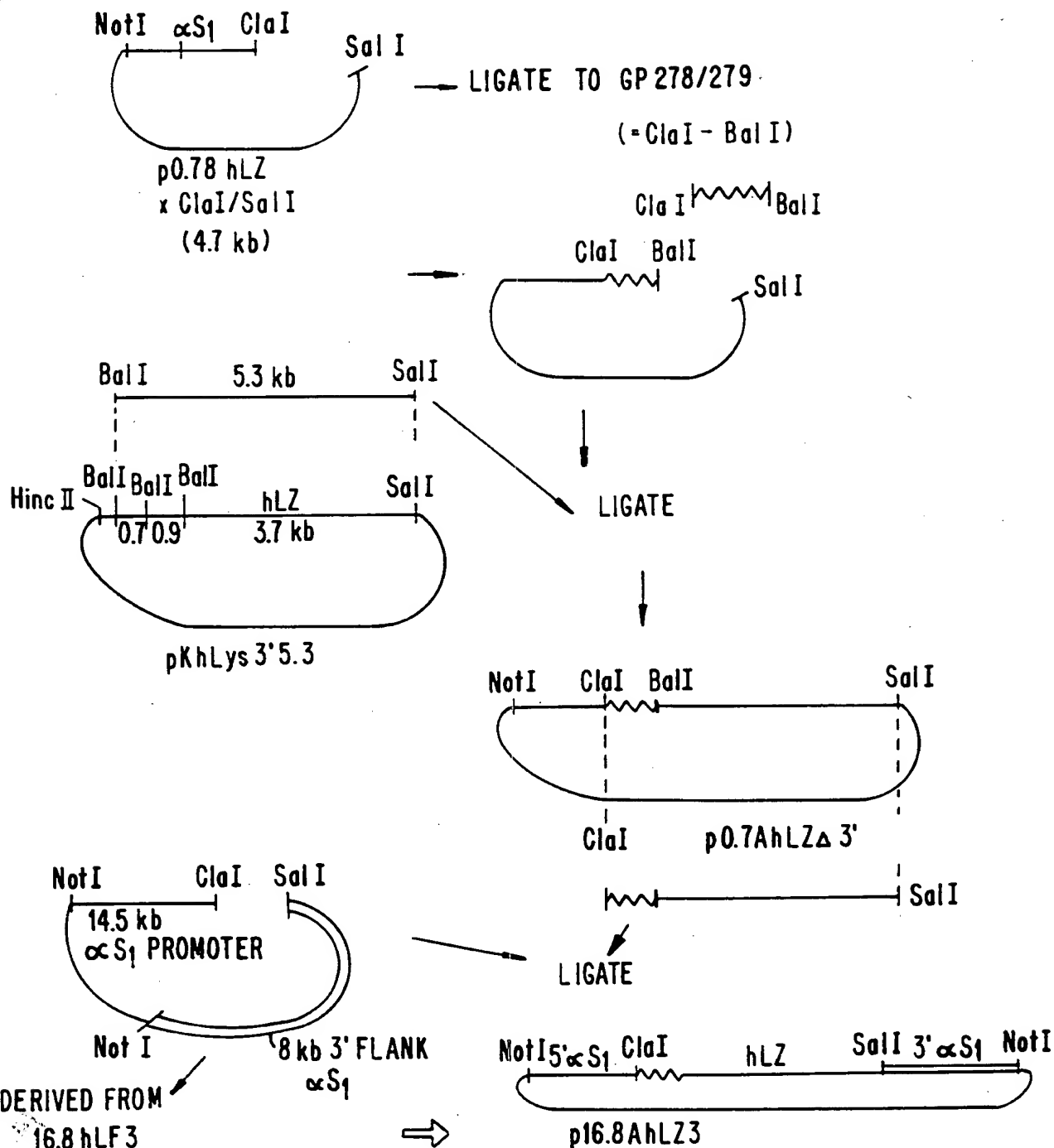


FIG. 26.

CONSTRUCTION OF 16 A hLZ3:

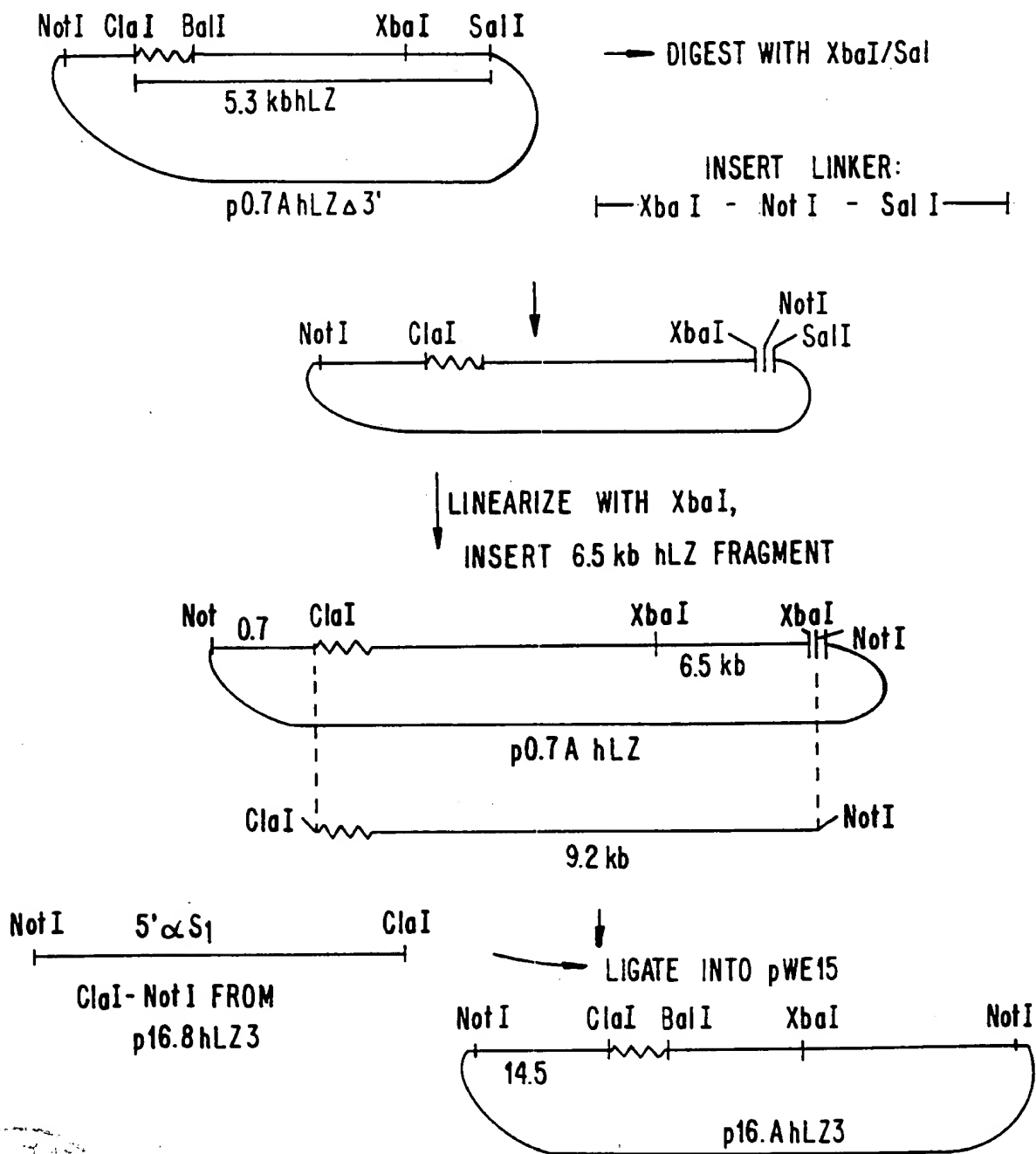


FIG. 27.